EVALUATION OF ETHANOLIC EXTRACTS OF *Dichrostachys glomerata* and *Psidium guajava* LEAVES FOR ANTIDIARRHOEAL ACTIVITY

BY

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JANUARY, 2008

DECLARATION

I hereby declare that this thesis titled **"Evaluation of Ethanolic Extracts of *Dichrostachys glomerata* and *Psidium guajava* Leaves for Antidiarrhoeal Activity"** has been written by me in the department of Veterinary Physiology and Pharmacology under the supervision of Prof. M. Mamman, Prof. J. O. Ayo and Dr. L. O. Mgbojikwe. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree at any university and it is a record of my own research work. It has not been presented in any previous application for a higher degree. All quotations and sources of information are specifically acknowledged by means of references.

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CERTIFICATION

This thesis entitled "Evaluation of Ethanolic Extracts of *Dichrostachys glomerata* and *Psidium guajava* Leaves for Antidiarrhoeal Activity" by Obi Jude, OKPARA meets the regulations governing the award of the degree of Master of Science, Ahmadu Bello University, Zaria and is approved for its contribution to scientific knowledge and literary presentation.

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This thesis is dedicated to Late Dr. L. O. Mgbojikwe (formerly of National Veterinary Research Institute, Vom) who participated actively in this work but died before its defence.

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ABSTRACT

To appraise some of the ethnomedical uses of *Psidium guajava* Lineaus (family: Myrtaceae) and *Dichrostachys glomerata* Forsskal (family: Leguminosae) the present work was undertaken to investigate the antidiarrhoeal properties of the ethanolic leaf extracts of these plants (*P. guajava* and *D. glomerata*). The acute oral toxicity studies of the extracts in mice, phytochemical screening of the extracts for bioactive principle, antibacterial, anti-inflammatory and anthelmintic actions were also studied. The orally determined LD_{50} values of the extracts in mice were found to be 1750.0 ± 212.0 mg/kg and 1600.0 ± 283.0 mg/kg for *P. guajava* and *D. glomerata*, respectively. The extracts were, thus, confirmed to be relatively toxic to mice. The phytochemical screening of the extracts revealed the presence of flavonoids, tannins, saponins, resins, triterpenoids, carbohydrates and reducing sugars.
The extracts (100, 200 and 400 mg/kg *i.p.*) caused a dose dependent significant ($P<0.05$) decrease of gastro-intestinal distance travelled by the charcoal plug in mice compared to control (Normal Saline) (5ml/kg *i.p.*) and markedly protected rats against Castor oil-induced diarrhoea with loperamide (5mg/kg *p.o.*) as reference antidiarrhoeal agent. The punch hole method was used for antibacterial screening of the extracts (50, 100 and 200 mg/ml) using pure cultures of *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*. The results were evaluated as zones of inhibition around the hole impregnated with varying concentrations of the extracts and ampicillin (10 mg/ml) as reference antibacterial drug. The zone of inhibition ranged between 8.07 – 16.07 mm for *P. guajava* and 10.12 – 20.00 mm for *D. glomerata* leaves extracts. Like indomethacin (10mg/*kg p.o.*) the extracts (200, 400 and 800 mg/kg *p.o.*) produced a dose and time related, sustained and significant reduction ($P<0.05$ – 0.001) in fresh egg albumen (0.5 ml/kg) induced acute inflammation of rat hind paw oedema. The ethanolic extracts of *P. guajava* and *D. glomerata* (200, 400, 800mg/kg *p.o.*), like albendazole also, significantly ($P<0.05$, $n = 5$) reduced the faecal egg count per gram (epg) of goats naturally infected with mixed gastro-intestinal nematodes. The percentage reduction ranged from 38 – 91% in *P. guajava* and 33 – 81% in *D. glomerata* treated goats, respectively. In *in vitro* studies, the extracts (0.4 – 3.2 mg/ml) significantly ($P<0.05$, $n = 3$) reduced the frequency and the amplitude of spontaneous pendular contractions of rabbit jejunum and the histamine – induced contraction of guinea pig ileum (a spasmolytic action). Atropine, an acetylcholine (ACh) blocker at the muscarinic receptors concentrations dependently reduced and or abolished spasmogenic effects of ACh. The effects of the extracts were equally compared with those of ACh and histamine (H) whose receptors are found in the gastro-intestinal tract. The effects of ACh (8.0×10^{-2}µg/ml) on the frequency and amplitude of contraction of the isolated rabbit jejunum and histamine (4.0×10^{-1}µg/ml) on isolated guinea pig ileum decreased ($P<0.05$) when combined with 3.2 mg/ml of the extracts of *P. guajava* and *D. glomerata*. The results suggested an antagonistic action between ACh and H_2 and the extracts. It is, therefore, postulated that the extracts of *P. guajava* and *D. glomerata* may have caused decrease in gastrointestinal propulsion through either anti-cholinergic effect and or H_2 – receptor system antagonism. These findings lend pharmacological support to the traditional use of *P. guajava* and *D. glomerata* leaves extract in the management and/or control of diarrhoea in man and his animals.

CONTENTS

TITLE PAGE … … … … … … … … … i

DECLARATION … … … … … … … … … ii

CERTIFICATION … … … … … … … … iii

DEDICATION … … … … … … … … … iv

ACKNOWLEDGEMENT … … … … … … … … v

ABSTRACT … … … … … … … … … vii

TABLE CONTENTS … … … … … … … … viii

LIST OF TABLES … … … … … … … … xiii

LIST OF FIGURES … … … … … … … … xv

LIST OF PLATES … … … … … … … … xvi

LIST OF APPENDICES … … … … … … … … xvii

LIST OF ABBREVIATIONS … … … … … … … … xix

LIST OF SYMBOLS … … … … … … … … xx

CHAPTER ONE

1.0 Introduction … … … … … … … … 1

1.1 Importance of Plants … … … … … … … … 1

1.2 Phytomedicines … … … … … … … … … 1

1.3 *Dichrostachys species* … … … … … … … … 3

1.4 *Psidium species* … … … … … … … … … 3

1.5 Aim and objectives … … … … … … … … 4

CHAPTER TWO

2.0 Literature Review … … … … … … … … … 5

2.1 Self-Medication in Animals … … … … … … … 5

2.2 Ethnoveterinary Pharmacology … … … … … … … 6

2.3 Ethnoveterinary Practices … … … … … … … … 9

2.4 Some Active Principles of Medicinal Herbs … … … … 11

2.5 The Role of Plant Secondary metabolites in Health Maintenance … 16

2.6 The Standard Drugs … … … … … … … 19

2.6.1 Acetylcholine … … … … … … … … 19

2.6.2 Atropine … … … … … … … … … 23

2.6.3 Histamine … … … … … … … … … 25

2.6.4 Imodium … … … … … … … … … 27

2.7.0 Taxonomy of *D. glomerata* (Forsskal) … … … … … 28

2.7.1 Synonyms … … … … … … … … … 28

2.7.3 Common name(s) … … … … … … … … 30

2.7.4 Local names … … … … … … … … 30

2.7.5 Geographical Range … … … … … … … 30

2.7.6 Habitat … … … … … … … … … 30

2.7.7 Description of *D. glomerata* … … … … … … 30

2.7.8 Products and Uses …… … … … … … … 31

2.7.9 Ethnomedical Uses … … … … … … … 32

2.8.0 Taxonomy of *P. guajava* (Linnaeus) … … … … … 33

2.8.1 Synonyms … … … … … … … … … 33

2.8.2 Common names … … … … … … … … 35

2.8.3 Description …… … … … … … … … 35

2.8.4 Geographical Range … … … … … … … 35

2.8.5 Tribal and Herbal Medicinal Uses … … … … … 35

2.8.6 Phytochemical and Biological Activities … … … … … 36

2.9.0 Diarrhoea … … … … … … … … … 39

CHAPTER THREE

3.0 . Materials and Methods … … … … … … … 43

3.1 Materials … … … … … … … … …. 43

3.2 Apparatus … … … … … … … … … 43

3.3 Chemicals, Test Substances and Physiological Salt Solution … … 43

3.4 Animals … … … … … … … … … 44

3.4.1 Study Animals … … … … … … … … 44

3.5 Test Organisms … … … … … … … … 45

3.6 Methods … … … … … … … … … 45

3.6.1 Collection, Drying and Grinding of *P. guajava* and *D. glomerata* leaves … … … … … … … 45

3.6.2 Preparation of Crude Ethanolic Extract from the Powdered Leaves of *P. guajava* and *D. glomerata.* … … … … … 46

3.6.3 Acute Oral Toxicity Studies of Crude Ethanolic Extract of *P. guajava* and *D. glomerata* Leaves in mice … … … …. 46

3.6.4 Pilot Acute toxicity Studies of *P. guajava* …. … …. …. 47

3.6.5 Determination of LD_{50} of Crude Ethanolic Extract of *P. guajava* Leaf in Mice … … … … … … … … 48

3.6.6 Determination of LD_{50} of Crude Ethanolic Extract of *D. glomerata* Leaf in Mice … … … … … … … … 48

3.6.7 Post-mortem Examination of mice Treated with Crude Ethanolic Extract of *P. guajava* and *D. glomerata* … … … … … 55

3.6.8 Phytochemical Tests … … … … … … … 55

3.6.9 Gastrointestinal Mobility Studies in mice … … …. … … 58

3.7.0 Studies on Castor –Oil-Induced Diarrhoea in Rats … … … 59

3.7.1 Antibacterial Screening of *P. guajava* and *D. glomerata* … … …. 60

3.7.2 Evaluation of Anti-inflammatory Property of *P. guajava* and *D. glomerata* Extract in Rats … …. … … … … 61

3.8.0 Isolated Tissue Studies … … … … … … … 63

3.8.1 Preparation of Solution for Isolated Tissue Studies … … … 63

3.8.2 Preparation of Rabbit Jejunum and Guinea Pig Ileum … … 63

3.8.3 Application of Test Substances to the Isolated Rabbit Jejunum and

Guinea Pig Ileum … … … … … … … … 63

3.8.4 Effects of the Crude Ethanolic Extracts of *P. guajava* and *D. glomerata* Leaves, Agonists and Antagonists on Isolated Rabbit Jejunum and Guinea Pig Ileum … … … … … 64

3.8.5 Evaluation of Anthelmintic Properties of *P. guajava* and *D. glomerata* Leaves Ethanolic Extracts … … … …. 65

3.8.6 Evaluation of Results … … … … … … … 66

CHAPTER FOUR

4.0 RESULTS … … … … … … … … 67

4.1.1 Preparation of Ethanolic Extract of *P. guajava* leaves … … … 67

4.1.2 Preparation of Ethanolic Leaf Extract of *D. glomerata* … … 67

4.1.3 Acute Oral Toxicity Studies of Crude Ethanolic Leaf Extract of *P. guajava* …… … … … … … … … 68

4.1.4 Acute Oral Toxicity Studies of Crude Ethanolic Leaf Extract of *D. glomerata* in Mice … … … … … … … 68

4.1.5 Gross Post-mortem Findings … … … … … … 73

4.1.6 Phytochemical Analysis … … … … … … … 73

4.1.7 Effect of Varying Concentrations of the Ethanolic Leaf Extracts of *P. guajava* and *D. glomerata* on contractile activity of Isolated Rabbit Jejunum … … … … … … … … 75

4.1.8 Contractile Response of Guinea Pig Ileum to varying Concentrations of Histamine and the Ethanolic Extracts of *P. guajava* and *D. glomerata* … … … … … … … … 85

4.1.9 Anti-inflammatory Screening … … … … … … 91

4.1.11 Effect of the Extract in Gastrointestinal Transit in Mice … … 96

4.1.12 Effect of the Extracts on Castor-Oil-Induced Diarrhoea in Rats …. 96

4.1.13 Antibacterial Efficacy of Leaf Extracts of *P. guajava* and *D. glomerata* … … … … … … … … 101

4.1.14 Anthelmintic Studies (Effects of the Extracts on Faecal egg count) … 107

CHAPTER FIVE

5.0. DISCUSSION, CONCLUSION AND RECOMMENDATION … 112

5.1 Discussion …… … … … … … … … 112

5.2 Conclusion …… … … … … … … … 119

5.3 Recommendation … … … … … … … … 120

REFERENCES … … … … … … … … 121

APPENDICES … … … … … … … … 138

LIST OF TABLES

TABLE		PAGE
1.0	Antidiarrhoeal Herbs … … … … … …	8
2.0	World Wide Uses of *P. guajava* Leaves and Root – bark ….	38
4.0	Result of the Pilot Test for LD_{50} of the Ethanolic Leaf Extract of *P. guajava* in Mice … … … … …	70
4.1	Test for Median Lethal Dose (LD_{50}) of Ethanolic Extract of *P. guajava* Leaf …… … … … … …	70
4.2	Determination of Median Lethal Dose (LD_{50}) of Ethanolic Leaf Extract of *P. guajava* … … … … …	71
4.3	Results of the Pilot Test for LD_{50} of Ethanolic Extract of *D. glomerata* … … … … … …	71
4.4	Test for LD_{50} of Ethanolic Leaf Extract of *D. glomerata* …	72
4.5	Determination of Median Lethal Dose (LD_{50}) of Ethanolic Leaf Extract of *D. glomerata* … … … …	72
4.6	Phytochemicals in the Crude Ethanolic Leaf Extract of *P. guajava* and *D. glomerata* … … … … …	74
4.7	The Inhibitory Effect of the Ethanolic Leaf Extract of *D. glomerata* (DGE: 0. 4 - 3. 2 mg/ml) and Atropine (1.3×10^{-1} µg/ml) in ACh (8.0×10^{-2} µg/ml) induced Contractions of the Rabbit Jejunum … … … …	82
4.8	Inhibitory Effect of the Ethanolic Leaf Extract of *P. guajava* (PGE: 0.4 – 3.2 mg/ml) and Atropine (1.3×10^{-1} µg/ml) in ACh (8.0 x 10-2 µg/mg) Induced Contractions of the Rabbit Jejunum … … …	83
4.9	Doses of Agonists Producing Maximal Dose Related Contraction Responses on Isolated Intestinal smooth Muscles and Concentration of Corresponding Antagonists Reverting those Actions … … … …	84
4.10	Effects of the Ethanolic Leaf Extract of *P. guajava* on Fresh Egg albumen – Induced Paw oedema in Rats …	92

4.11 Effect of Ethanolic Leaf Extract of *D. glomerata* on Fresh Egg Albumen – Induced Paw Oedema in Rats … … … 94

4.12 Effect of the Ethanolic Leaf Extract of *D. glomerata* on Gastrointestinal transit in Mice … … … … … 97

4.13 Effect of the Ethanolic Leaf Extract of *P. guajava* on Gastrointestinal Transit in Mice … … … … … 98

4.14 Effect of the Ethanolic Leaf Extract of *D. glomerata* on Castor Oil (0.5ml/Rat) – Induced Diarrhoea in Rats … … 99

4.15 Effect of the Ethanolic Leaf Extract of *P. guajava* on Castor Oil (0.5ml/Rat) – Induced Diarrhoea in Rats … … … 100

4.16 Antibacterial Zone of Inhibition of Ethanolic Leaf Extract of *P. guajava* … … … … … … … 102

4.17 Antibacterial Zone of Inhibition of Ethanolic Extract of *D. glomerata* … … … … … … … 103

4.18 Faecal Egg Count Per Gram (epg) and Percentage Reduction (%) of Faecal Egg Count for *P. guajava* Leaf Extract Treated Goats Compared with Untreated (control) Group … … … … … … … 108

4.19 Faecal Egg Count Per Gram (EPG) and Percentage Reduction (%) of Faecal Egg Count for *D. glomerata* Leaf Extract Treated Goats Compared with Untreated (control) Group … 109

LIST OF FIGURES

FIGURE		PAGE
2.0	Chemical Structure of Acetylcholine … … … … …	22
2.1	Chemical Structure of Atropine … … … … … …	24
2.2	Chemical Structure of Histamine … … … … … …	26
4.0	Normal Spontaneous Pendular Contraction of Rabbit Jejunum … …	76
4.1	Effects of Acetylcholine (ACh) on Spontaneous Contraction of Isolated Rabbit Jejunum … … … … … …	77
4.2	Concentration-Dependent Effects of *Psidium guajava* Leave Extract on the Frequency and Amplitude of Contraction of Isolated Rabbit Jejunum … … … … … …	78
4.3	Concentration-dependent Effects of *Dichrostachys glomerata* Leaf Extract on the Frequency and Amplitude of Contraction of Isolated Rabbit Jejunum … … … … … …	79
4.4	Effects of Atropine and Acetylcholine on the Spontaneous Pendular Contraction of Isolated Rabbit Jejunum … … … … …	80
4.5	Inhibitory Effects of *P. guajava* and *D. glomerata* on Acetylcholine induced contraction of Isolated Rabbit Jejunum … … … …	81
4.6	Normal Contraction of Isolated Guinea Pig Ileum … … …	86
4.7	Histamine-Induced contractions of Isolated Guinea Pig Ileum …	87
4.8	Effects of *Psidium guajava* Leaf Extract on Histamine-Induced Contraction of Isolated Guinea Pig Ileum … … …	88
4.9	Effects of *Dichrostachys glomerata* Leaf Extract on Histamine-Induced Contraction of Isolated Guinea-pig Ileum …	89
4.10	Inhibitory Effects of Mepyramine on Histamine induced contraction of isolated Guinea-Pig Ileum … … … … …	90
4.11	Effects of Ethanolic Leaf Extract of *P. guajava* on Fresh Egg Albumen–induced Paw Oedema in Rats … … … … …	93
4.12	Effects of Ethanolic Leaf Extract of *Dichrostachys glomerata* on Fresh Egg Albumen–induced Paw Oedema in Rats … … …	95
4.13	Effects of *Psidium guajava* Leaf Extract on Feacal Egg Count per gram (epg) Compared with the Control (Albendazole) … …	110
4.14	Effects of *Dichrostachys glomerata* Leaf Extract on Faecal Egg Count per gram (epg) Compared with the Control (Albendazole) …	111

LIST OF PLATES

PLATE		PAGE
2.0	*Dichrostachys glomerata* (Forsskal) Plant and Flower … … …	29
2.1	*Psidium guajava* (Linnaeus) Plant … … … … …	34
3.0	Air Dried Leaves of *Psidium guajava* … … … … …	50
3.1	Powdered Leaves of *Psidium guajava* … … … … …	51
3.2	Air Dried Leaves of *Dichrostachys glomerata* … … … …	52
3.3	Powdered Leaves of *Dichrostachys glomerata* … … … …	53
3.4	Soxhlet Extractor … … … … … … … … …	54
4.0	Inhibition of the Growth of *Staphylococcus aureus* by Different Concentration of *Dichrostachys glomerata* and *Psidium guajava* Leaves Ethanolic Extracts …… … … … … …	104
4.1	Inhibition of the Growth of *Salmonella typhi* by Different Concentrations of *Dichrostachys glomerata* and *Psidium guajava* Leaves Ethanolic Extracts …… … … … … …	105
4.2	Inhibition of the Growth of *Escherichia coli* by Different Concentration of *Psidium guajava* and *Dichrostachys glomerata* Leaves Ethanolic Extracts …… … … … … …	106

LIST OF APPENDICES

APPENDIX		PAGE
1.1	Faecal Egg Count Per Gram (epg) for *Dichrostachys glomerata* Leaf Extract Treated Goats (Day O, Pre-treatment n = 5) … …	138
1.2	Faecal Egg Count Per Gram (epg) for *D. glomerata* Leaf Extract Treated Goats (Day 6, Post-treatment … … … … …	139
1.3	Faecal Egg Count Per Gram (epg) for *D. glomerata* Leaf Extract Treated Goats (Day 12; Post-treatment) … … … … …	140
1.4	Faecal Egg Count Per Gram (epg) for *D. glomerata* Leaf Extract Treated Goats (Day 18; Post-treatment) … … … … …	141
1.5	Faecal Egg Count Per Gram (epg) for *D. glomerata* Leaf Extract Treated Goats (Day 24; Post-treatment) … … … … …	142
1.6	Faecal Egg Count Per Gram (epg) for *D. glomerata* Leaf Extract Treated Goats (Day 30; Post-treatment) … … … … …	143
1.7	Faecal Egg Count Per Gram (epg) for *D. glomerata* Leaf Extract Treated Goats (Day 36; Post-treatment) … … … … …	144
2.1	Faecal Egg Count Per Gram (epg) for *Psidium guajava* Leaf Extract Treated Goats (Day 0; Pre-treatment) … … … … …	145
2.2	Faecal Egg Count Per Gram (epg) for *Psidium guajava* Leaf Extract Treated Goats (Day 6; Post-treatment) … … … … …	146
2.3	Faecal Egg Count Per Gram (epg) for *P. guajava* Leaf Extract Treated Goats (Day 12; Post-treatment) … … … … …	147
2.4	Faecal Egg Count per Gram (epg) for *P. guajava* Leaf Extract Treated Goats (Day 18; Post-treatment) … … … … … …	148
2.5	Faecal Egg Count per Gram (epg) for *P. guajava* Leaf Extract Treated Goats (Day 24; Post-treatment) … … … … …	149

2.6 Faecal Egg Count per Gram (epg) for *P. guajava* Leaf Extract Treated Goats (Day 30; Post-treatment) … … … … … 150

2.7 Faecal Egg Count per Gram (epg) for *P. guajava* Leaf Extract Treated Goats (Day 36; Post-treatment) … … … … … 151

3.1 Effects of Distilled Water (5ml/kg) on Fresh Egg Albumen-Induced Paw Oedema in Rats (n = 8) … … … … … … 152

3.2 Effects of *Psidium guajava* Leaf Extract (200mg/kg) on Fresh Egg Albumen-Induced Paw Oedema in Rats (n = 8) … … … … 153

3.3 Effects of *P. guajava* Leaf Extract (400mg/kg) on Fresh Egg Albumen- Induced Paw Oedema in Rats (n = 8) … … … … 154

3.4 Effects of *P. guajava* Leaf Extract (800mg/kg) on Fresh Egg Albumen- Induced Paw Oedema in Rats (n = 8) … … … 155

3.5 Effects of Indomethacin (10mg/kg) on Fresh Egg Albumen- Induced Paw Oedema in Rats (n = 8) … … … … … … 156

3.6 Effects of *Dichrostachys glomerata* Leaf Extract (200mg/kg) on Fresh Egg Albumen-Induced Paw Oedema in Rats (n = 8) … … … 157

3.7 Effects of *D. glomerata* Leaf Extract (400mg/kg) on Fresh Egg Albumen-Induced Paw Oedema in Rats (n = 8) … … … … 158

3.8 Effects of *Dichrostachys glomerata* Leaf Extract (800mg/kg) on Fresh Egg Albumen-Induced Paw Oedema in Rats (n = 8) … … … 159

LIST OF ABBREVIATIONS

ABU	-	Ahmadu Bello University
ACh	-	Acetylcholine
AChE	-	Acetylcholinesterase
BDH	-	British Drug House
Ca^{2+}	-	Calcium ion
$CaCl_2$	-	Calcium chloride
Dg	-	*Dichrostachys glomerata*
DGE	-	*Dichrostachys glomerata* extract
EC	-	Effective concentration
et al	-	And others
H	-	Histamine
H_2	-	Histamine receptor
ip	-	Intraperitoneal
K^+	-	Potassium ion
KCl	-	Potassium chloride
MCC	-	Maximum convenient concentration
MCV	-	Maximum convenient volume
Na^+	-	Sodium ion
NaCl	-	Sodium chloride
$NaHCO_3$	-	Sodium bicarbonate
$NaH_2PO_4.2H_2O$	-	Sodium dihydrogen phosphate
NAPRI	-	National Animal Production Research Institute
NVRI	-	National Veterinary Research Institute
O_2	-	Oxygen
PGE	-	*Psidium guajava* Extract
p. o.	-	*per os*
W	-	Wash

LIST OF SYMBOLS

µ	-	Micron
µg	-	Microgram
±	-	Plus or minus
<	-	Less than
>	-	Greater than
≥	-	Greater or equal to
≤	-	Less or equal to

CHAPTER ONE

1.0 INTRODUCTION

1.1 Importance of Plants

From the earliest times, man acquired knowledge of the beneficial and adverse effects of plants from observations on animals. The health, growth and performance of animals, especially livestock, are affected by the chemical composition of the plants they consume. To distinguish edible from poisonous plants, grazing animals were observed and the plants not eaten were considered poisonous and were tried on beasts of prey (Nwude and Ibrahim, 1980). Plants that were observed to have deleterious effects on animals were avoided whereas those with beneficial effects on animals were made use of by man. Plants are generally studied either for their beneficial or toxicological effects (Nwude, 1997; Familluyi *et al.*, 2001; Githiori *et a.*, 2003; Dzenda, 2004).

Statistics from the World Health Organisation (WHO) shows that healing plants are the primary medicines for over two-third of the world's estimated 4.5 billion people. The potential of medicinal plant research results in animal and human health care scheme is no longer in doubt having gained recognition in several nations of the world and WHO (Odeola, 1998; Githiori *et al.*, 2003).

1.2 Phytomedicines

Despite the immense technological advancement in modern medicine, many people in Africa (approximately 75% of its population) still rely on traditional healing practices and medicinal plants for their daily health care needs. The floral biodiversity of Africa provides the African traditional medical practitioners with an impressive 'natural pharmacy', from which plants are selected as remedies, or as ingredients to prepare

herbal medicines (Phytomedicines) for an array of human and animal disorders (Mbi and Bilikha, 1998; Oyewole, 2003).

Although in Nigeria, there are no professional traditional veterinary practitioners as such, herdsmen, village elders and others, who kept animals have experience in diagnosing and treating animal diseases. Sometimes the same herbs used in treating human diseases are also used in treating corresponding animal diseases. However, there are some herbs that are used mainly for veterinary purposes and some diseases peculiar to animals (Nwude and Ibrahim, 1980; Nwude, 1997; Eruvbetine *et al.*, 1998).

Traditional veterinary knowledge and beliefs are commonly referred to as ethno-veterinary practice (EVP). Traditional remedies can be effective and at times scientists have acknowledged that they work on sound scientific reasoning.

Minga (1989) and Nwude (1997) in studies conducted in Tanzania and Nigeria, respectively, revealed that a number of diseases can be cured by local medicinal plants. The efficacy of plant extracts is due to their content of one or more biologically active principle (s). Pharmacological assays have shown that the activity is not always due to the major components, but the minor ones, or even to the synergism of all the principles (Oyewole, 2004).

Herbal medicinal products are assuming greater roles in the lives of people across the world in the face of global upsurge of drug resistance, toxicity, adverse effects and escalating costs of synthetic products. They are still the first line treatment for many of the word's population, being readily available, traditional and relatively inexpensive (Odeola, 1998; Olaniyi, 1998; Eno and Azah, 2004).

1.3 *Dichrostachys species*

Dichrostachys glomerata (Forsskal) Chior; belongs to the family *Mimosaceae.* The plant genus *Dichrostachys* is a shrub or small tree of about 8m tall. All the Nigerian *Leguminosae* have bi-pinnate alternate leaves, a character shared only with Moringa and a few genera of *Caescalpiniaceae*, with from one to about twenty five pairs of pinnae. The leaflets varying from one to sixty pairs are often very small and closely crowded and most genera are opposite (Dalziel, 1937). The stipules in some genera are modified into spines. The fruits are mostly elongated pods, fleshy, wooly, leathery or papery, containing several seeds and splitting open in the majority of cases. *Dichrostachys glomerata* (Sickle pod) is known as "dundu" in Hausa, "burli" in Fulani, "Kara" in Yoruba and "ami ogwu" in Igbo. The barks or leaves are pounded and macerated in cold water, spices and lime juice are added and the mixture given for the treatment of both dysentery and helminthosis in humans and animals (Dalziel, 1937; Cowan, 1998).

1.4 *Psidium species*

Psidium guajava (Linnaeus) belongs to the family *Myrtaceae* called guayaba in Spanish-speaking countries, guava in English and goiaba in Brazil. Guava is a common shade tree or shrub in door-yard gardens in the tropics. Cultivated varieties average about 10 meters in height and produce lemon-sized fruits. The tree is easily identified by its distinctive thin, smooth, copper-coloured bark that flakes off, showing a greenish layer beneath with elliptical-opposed leaves and white flowers. Guava is one of the richest fruit sources of vitamin C, since some of its varieties have five times the amount of the vitamins found in orange (Oliver-Bever, 1986; Iwu, 1993). The leaves and roots have been used for ages to treat diarrhoea and dysentery (Dalziel, 1937).

1.5 Aim and objectives of the Study

Aim

The Evaluation of ethanolic extracts of *Dichrostachys glomerata* and *Psidium guajava* leaves for antidiarrhoeal activity.

Objectives

- To ascertain the phytochemical constituents of *D. glomerata* and *P. guajava* leaf extracts.
- To ascertain the effects of the extracts on isolated rabbit jejunum and guinea pig ileum.
- To ascertain the effect of the extracts on acetylcholine and histamine–induced contraction of rabbit jejunum and guinea pig ileum respectively
- To ascertain the anti-inflammatory activity of the extracts on fresh egg albumin–induced rat paw oedema
- Determine the effect of *D. glomerata* and *P. guajava* on gastro-intestinal transit in mice
- Determine the effect of *D. glomerata* and *P. guajava* extracts on castor oil – induced diarrhoea in rats
- Determine the *in vitro* antimicrobial properties of the extracts of *D. glomerata* and *P. guajava*
- Determine the anthelmintic activity of the extracts on goats naturally infected with gastrointestinal worms
- Determine the oral median lethal doses (LD_{50}) of the extracts in mice.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Self-Medication in Animals

It has been observed that in the tropical environment animals reared under extensive husbandry system thrive better and are usually able to withstand some of the endemic diseases, to which intensively managed animals continually remain susceptible.This apparent disease resistance has been attributed, at least in part, to adequate exercise and the possibility of selective and unrestricted grazing whereby a wide variety of edible forages are available to the animals. Some of such plants have been shown to provide not only nutritional but also medicinal benefits (Burkill, 1985; Huffman, 1997; Adebayo *et al.*, 2001; Adedapo *et al.*, 2002). A growing body of evidence has accumulated regarding the phenomina of self-medication in animals (Huffman, 1997; 2001; 2002). A combination of biological evolution and selective pressure has acted upon hosts and their pathogens in such a way that animals have consequently, adopted behavioural strategies to counteract the deleterious effects of pathogens (Olaniyi, 1998; Cousin and Huffman, 2002).

A variety of non-nutritional plant secondary compounds are found in the diet of animals, but little is known about the possible medicinal consequences of their ingestion. One of the challenges of interpreting self-medication in animals is to distinguish between possible indirect medical benefits derived from secondary compound rich plants that are assumed to be ingested for their nutritional value versus limited and situation specific ingestion of items that are processed solely for their curative value or other physiological effects (Etkin, 1996; Cousin and Huffman, 2002).

In Bwiti legends the pygmies are said to have found *Tabernanthe iboga* (*Apocynaceae)*, however, it is possible that the pygmies themselves discovered the properties of the plant by watching wild boars digging up and eating the roots, only to go into wild frenzy, jumping around and fleeing from perhaps frightening images. Similar behaviour has been reported by indigenous peoples for porcupines and gorillas who are said to be fond of the roots (Pope, 1969). In South-East Cameroon gorillas eat the flowers and branches of Iboga (Butzlv, 1980), while in Gabon they eat the fruits, stem and roots of the plant (Valker, 1931). There is ample evidence that man, globally, has discovered the effects of drug plants by observing the behaviour of animals (Diurno *et al.*, 1996; Huffman, 2001; 2002).

2.2. Ethnoveterinary Pharmacology

Traditional medicine as practised in many parts of the developing countries was based partly on observations of adverse and beneficial effects of plants on animals and has undergone experimentation for generations (Nwude, 1997). It would be a pity if such experiences are abandoned entirely by the present and future generations. A research into the efficacy of herbs used in traditional veterinary practice would therefore be worthwhile (Ibrahim, 1996; Nwude, 1997). It is important to supplement modern drugs with effective local herbs, not only for economic reasons, but also because there is acute shortage of drugs from time to time. If the veterinary practitioner has effective local herbs at his disposal, he could use them on occasions when effective modern drugs are not available or where the local herbs prove more effective, cheaper or safer (Ekpendu *et al.,* 1998a, b; Cousin and Huffman, 2002; Oyewole, 2003, 2004).

The current economic hardship in the country makes it mandatory to explore all local resources for the provision of necessities of life. This includes the provision of drugs in

general and veterinary pharmaceuticals in particular. Raw materials for pharmaceutical abound in vegetable, animal and mineral sources (Ibrahim, 1996; Nwude, 1997).

Our vegetation being of the luxuriant tropical type, plant materials are in abundance all the year round and our people have depended almost entirely on them for generations for the provision of both human and veterinary drugs (Nwude, 1986; Ibrahim and Abdu, 1992). The materials include medicinal plant parts (leaves, barks, flowers and roots), and products (sugar, oil, e.g. groundnut oil, palm oil, cotton seed oil, castor oil, palm kernel oil, corn oil, shear butter oil) (Nwude, 1997; Huffman, 2001; 2002).

A lot of work has been done in this country on medicinal plants used in humans but not much has been done on those used in animals. Nwude and Ibrahim (1980) compiled information on plants used in traditional veterinary medicine. Along this line, Ibrahim *et al* (1984) investigated a number of plants used traditionally for the treatment of human and animal helminthosis and found the following plants to be effective against *Nippostrongylus braziliensis* in rats - *Aloe barteri, Angeissus leiocarpus, Annona senegalensis, Cassia occidentalis, Diospyros mespilformis and Terminalia avicennioides.* The methanolic extract of *Morinda lucida* and aqueous extract of *Alstonia boonie* bark each demonstrated activity against *Trypanosoma brucei* in mice (Asuzu and Chineme, 1990; Asuzu and Anaga, 1991). Also, Abdullahi *et al* (2001a) reported on the antidiarrhoeal activity of *Terminalia avicennioides* roots extract, while Okpara *et al., (*2006) evaluated *Adansonia digitata* leaves ethanolic extract for antidiarrhoeal property. Examples of plants used in traditional veterinary practice for the management of diarrhoea and related conditions are shown in Table 1:

Table 1.0: Antidiarrhoeal Herbs

S/No.	Family	Species	Part(s) used	References
1.	*Acanthaceae*	*Crossandra guineansis*	Leaves	Bonquet *et al.* (1971); Nishihara (1995)
		Thomandersia laurifolia	Leaves and roots	Tutin and Fernandez (1985)
2.	*Annonaceae*	*Annodium manni*	Stem-bark	Tutin and Fernandez (1985
3.	*Apocynaceae*	*Landolphia owariensis*	Twigs, stem and Leaves	Burkill (1985); Iwu (1993)
		Pysnobotrya nitida	Leaves and fruits	Bonquet *et al*. (1971)
		Rauwolfia macrophylla	Fruits	Sabater-Pi (1977)
		Tabernaemontana crassa	Stem-bark	Oliver-Bever (1986)
4.	*Bombacaceae*	*Adansonia digitata*	Leaves	Okpara *et al.* (2006).
5.	*Bromelisceae*	*Ananas comosus*	Fruits and leaves	Burkill (1985); Iwu (1993).
6.	*Caesalpiniaceae*	*Detarium macrocarpum*	Fruits and leaves	Yamagiwa *et al.*, (1994).
7.	*Cecropriaceae*	*Myrianthus aboreus*	*Leaves, stem and roots*	*Burkill, 1985*
8.	*Chrysobalanacea*	*Parinari excelsa*	*Leaves, and stem*	Yamagiwa *et al*., (1994).
9.	*Combretaceae*	*Quisqualis latiolata*	*Bark*	*Iwu (1993)*
		Terminalia avicennioides	*Roots*	Burkill (1985); Bonquet *et al.* (1971)
10.	*Convulvullaceae*	*Ipomoea involucrate*	*Leaves and stem*	Akubundu and Agyakwa (1987).
11.	*Euphorbiaceae*	*Alchornia cordfolia*	*Leaves, stem and roots*	Bonquet *et al*. (1971); Iwu (1993).
		Alchornia florbinda	*Roots and fruits*	Yamagiwa *et al*. (1994); Sabartar-Pi (1971)
12.	*Euphorbiaceae*	*Macaranga kilimandschar*	*Roots*	Tutin and Fernandez (1985)
		Tetracarpidium conophoru	*Leaves and fruits*	*Okerulu and Chinwe, (2001)*
13.	*Gramineae*	*Pennisetum purpureum*	*Leaves*	*Iwu (1993); Nishihara, (1995)*
14.	*Guttiferae*	*Garcina kola*	*Seeds, stem and root ba*	Bonquet *et al.*, (1971)
		Mammea africana	*Fruits, leaves and bark*	*Sabater (1977); Rogers et al. (1990).*
15.	*Icacinaceae*	*Lodes agricana*	Leaves	Yamagiwa *et al*., (1994); Bonquet *et al.*, (1971
16.	*Leguminosae*	*Cassia fistula*	Leaves, bark and flower	Bahorum *et al.*, 2005
17.	*Loganiaceae*	*Anthocleista vogelli*	*Stem, bark and leaves*	Rogers *et al*. (1990)
18.	*Minosoideae*	*Parkis filicoldea*	*Fruits and leaves*	Iwu (1993)
		Pentaclethra macrophylla	Bark, fruits and leaves	Iwu, (1993); Bonquet *et al*. (1971)
19.	*Musaceae*	*Musa paradisiacal*	Fruits and leaves	Iwu (1993)
20.	*Palmae*	*Anastrophyllum secudiflor*	Shoots and saps	Iwu (1993)
21.	*Passifloraceae*	*Barteria nigritiana*	Fruits and leaves	Iwu (1993)
22.	*Rubiaceae*	*Porterandia cladantha*	*leaves*	Iwu, (1993), Nishihara (1995)
23.	*Sapindaceae*	*Allophyllus africanus*	Fruits, leaves and roots	Iwu, (1993), Nishihara, (1995)
24.	*Ulmaceae*	*Celtis brieyi*	*Leaves, fruits and roots*	Bonquet *et al.* (1971)
25.	*Zingiberaceae*	*Costus lucanusianus*	*Rhizomes*	*Nishihara (1995)*

2.3. Ethnoveterinary Practices

Traditional veterinary knowledge and or beliefs are commonly referred to as ethnoveterinary practices (EVP) (Eruvbetine *et al.*, 1998). Traditional remedies can be highly effective and at times scientists have acknowledged that they work on sound scientific reasoning. However, some of the problems associated with traditional medicine include the lack of scientific proof, unpredictable dosage, intangible practices of traditional medicine and possible improper diagnosis of ailment (Dalziel, 1937).

The mystic rites that surround human traditional medicine and that meet with objections of modern orthodox medical practitioner are virtually non-existent in traditional veterinary medical practice (Nwude, 1997). There are some uses of herbs in traditional veterinary medicine that are based on superstition or on the "laws of signature" that would make the modern veterinary practitioners suspicious of their effectiveness. For example, *Ficus capensis* is used to improve fertility because it has abundant clustered fruits and many plants with milky juices are used to increase lactation (Nwude, 1997). On the other hand, there is no doubt that some of the remedies would be effective. For example, *Pausinystalia yohimbe* has a reputation on the Cameroons as an aphrodisiac. It has been found to contain the alkaloid yohimbine that is used in modern veterinary medicine as aphrodisiac. *Myracrodruon urunaleava* Allemao *(Anacardiaceae)* is used in traditional medicine as antidiarrhoeal agent. Chalcones and dihydrochalcones isolated from the plant showed hepatoprotective, antidiarrhoeal and anti-ulcer activities in experimental models (Viana *et al.*, 1997). Also, *Nicotiana tabacum* used in traditional medicine as insect repellant in horses and insecticide in poultry has been found to contain nicotine known to have insecticidal properties (Nwude, 1997).

In ethnoveterinary practice, diseases are usually distinguished by means of clinical signs, epizootiology and in few cases attributed to supernatural causes. Failure to explain the principle of Ethnoveterinary science has caused considerable misunderstanding between pastoralists and veterinarians. It is, however, argued that investigation should be carried out to find out ways of cultivating and harvesting medicinal plants useful in the treatment of specific diseases and efforts made to integrate the existing knowledge into modern animal healthcare programme (Carroll, 1980; Eruvbetine *et al.,* 1998).

In recent times, there has been an increased awareness of the importance of traditional medicine in the healthcare of human and animal populations in developing countries. Efforts are being made in different countries to carry out research into traditional medicine and to integrate it with modern orthodox medicine (Burkill, 1985; Adedapo *et al.,* 2002). The use of traditional medicine has increased among others due to high cost of veterinary drugs, poor efficacy of adulterated synthetic drugs sold in the market and the lack of veterinary services, especially in isolated pastoral areas (Wanyama, 1997; Githiori *et al.,* 2003).

According to Dilhuydy (2003), the practice of complementary and alternative medicine is equally on the increase world wide because of its effectiveness in the management of terminal diseases and other ailments, which are refractory to orthodox medicine and the general belief that traditional or herbal medicines are less toxic.

In Nigeria, just as in many other African countries like Kenya, Cameroon and Tanzania, plant extracts and products are employed in traditional veterinary practice to treat many ailments (Satoh *et al.,* 2001). According to Ibrahim *et al* (1984), the concept of animal disease and treatment traditionally held by herdsmen is often close to the orthodox.

Cattle diseases are caused by infectious agents, trauma, *etc*. For instance "goli" is a disease of calves characterized by constipation or diarrhoea and poor growth caused by worms. "Goli" is translated by field veterinarians as helminthosis (Nwude, 1997).

McCorkle and Mathias-Muddy (1992) reported on Ethnoveterinary practice in Africa as follows. "Vaccination": Many pastoral societies in Africa, including Nigeria, long ago invented their own vaccination for infectious diseases. Effective vaccinations for contagious bovine pleuropneumonia (CBPP), foot-and-mouth disease (FMD) and bovine brucellosis have been developed".

"Surgery: All stock raising peoples have appropriate methods of wound care. Nigerian Fulanis have demonstrated dramatic surgical skills as when they successfully replaced the unperforated intestines of a gored animal".

"Dietary supplementation: Stock owners always endeavour to provide their animals with supplementary minerals, edible earth, special feeds and other nutritious items to forestall illness, enhance appetite, growth, libido, fertility, etc".

Research into Ethnoveterinary pharmacology and Ethnoveterinary practice is necessary to salvage what remains of our indigenous knowledge of veterinary medicine, and conscious efforts must be made to integrate Ethnoveterinary medicine with modern veterinary medicine (Nwude, 1997).

2.4. Some Active Principles of Medicinal Herbs

The isolation of the constituents of medicinal plants has been practiced for many years. It commenced with the isolation of opium alkaloids in the early 19^{th} century and has resulted in the discovery of a large number of compounds with a very wide diversity of

structure and biological activities. The last fifty years have seen much activity in this area and many thousands of novel substances have been isolated and characterized. This has been largely assisted by the introduction and application of chromatographic and advanced spectroscopic techniques (Houghton, 1998). With modern advances in the techniques for isolation and structure determination of active principles, even minute amounts of them can be isolated and their structures determined (Salemink, 1980).

The efficacy of herbal extracts is due mainly to the presence of one or more biologically active principles. Pharmacological assays have demonstrated that activity is not always due to the main compounds, but the minor ones or even the synergism of all the active principles (Galeffi, 1980). Medicinal plants are the most ancient source of drugs for curing human and animal diseases. Their recognized biological actions led to their cultivation even in antiquity, in Egypt, Greece, China and along the Mediterranean. Experience gained over thousands of years, thus, led to the techniques of cultivation and collection of medicinal herbs of today. About one-quarter of all medicines are obtained from the 250,000 flowering plants on earth's surface (Farkas, 1980; Balick and Cox, 1996; Ekpendu *et al.*, 1998 a, b).

Nigeria is naturally endowed with a wide variety of vegetation ranging from the thick rainforest in the South to the sparse vegetation in the North. Of recent many scientists have focused attention on a number of these tropical trees and plants in search of novel active compounds (Fagbule and Olatunji, 1984; Wambebe *et al.*, 1986; Asuzu and Anaga, 1991; Sofowora, 1993; Haruna and Choudhury, 1997; Abatan *et al.*, 1997; Nwude, 1997; Choudhury *et al.*, 1997; Olaleye *et al.*, 2004; Oyewole, 2004; Eno and Azah, 2004*)*.

Sofowora (1993) analysed the chemical constituents of the stem-bark extract of *Mitragyna rubrostipulata* which showed the presence of mitraphylline, an alkaloid used as a remedy for dysentery and diarrhoea in some parts of Nigeria. *Carica papaya* (Pawpaw) seeds are used by traditional medicine practitioners in Northern states of Nigeria as a general vermifuge and antidiarrhoeal agent (Lal and Gupta, 1976; Wambebe *et al.,* 1986). Benzol thiocyanate is formed when the seeds of pawpaw are macerated in water or organic solvent (Wambebe *et al.,* 1986). The biologically active components of *Carica papaya* seeds have been shown to be active against bacteria, yeast and moulds (El-Tayeb *et al.,* 1974), *Hymenolepis nana* (Katiyer and Sen, 1971) and *Nippostrongylus braziliensis* in rats (Wambebe *et al.,* 1986).

Similar reports came from Olaleye *et al* (2004) who attributed the antioxidant and anti-inflammatory properties of the leaves of *Voacanga africana* to its flavonoid and tannin constituents. Oyewole (2004) also reported the presence of flavonoids and tannins in *Sclerocarya birrea* stem-bark. This plant is reputed for its antidiarrhoeal, anti-inflammatory and antimalarial properties (Oyewole, 2004). The antimicrobial effect of *Tetracarpidium conophorum* extracts were evaluated by Okerulu and Chinwe (2001), and they attributed the inhibitory action of the extracts on *Streptococcus viridans* and *Staphylococcus epidermidis* to the presence of anthraquinones, tannins and flavonoids. The red and white varieties of *Ipomoea involucrate* are used as antidiarrhoeal agents ethnomedically. The abundance of flavonoids in the ethanolic and petroleum ether extracts of the leaves was linked to the observed biological activity (Ejimadu and Ogbeide, 2001).

Flavonoids are good scavengers of free radicals - hydroxyl radicals (OH), hydrogen peroxide (H_2O_2), superoxide anion (O^{2-}), nitric oxide (NO) and peroxynitrite $(ONOO)^-$ -

which perpetrate brain injury through oxidative modification of DNA, proteins, lipids, amino acids and loss of blood brain barrier integrity *in vivo* (Ischiropoulus and Beckman, 2003*;* Iwalokun *et al.,* 2004). Brain cell which consumes one-fifth of daily body oxygen requirements (Chan, 1996), is rich in polysaturated fatty acids but limited in the levels of antioxidant enzymes (Floyd *et al.,* 1990). It is, therefore, not surprising that accelerated brain damage and neurodegeneration were observed in animal models in which free radicals formed the pathogenic bases for premature ageing (Hertog *et al.,* 1994).

The compounds present in *Tephrosia vogelli* used ethnomedically as molluscide, purgative and insecticide (Chiu, 1998) and for tooth-ache remedy (Watt and Breyer-Brandwijk, 1962) include flavonoids, glycosides, steroids, tannins and reducing sugars in the leaves (Ekpendu *et al.,* 1998b). Rotenone, a flavonoid is the compound of primary interest in *Tephrosia vogelli* as far as its insecticidal property is concerned (Lambert *et al.,* 1993; Tomlin, 2000).

Menezes (1998) showed that aqueous as well as ethanolic extracts from *Myracrodruon urundeava* Allemao *(Anacardiaceae)* exert potent anti-inflammatory activity in experimental models of acute and sub-acute inflammation. The hydroalcoholic extract also showed hepatoprotective antidiarrhoeal and anti-ulcer properties. Chalcone and catechnic tannins are implicated in the pharmacological activity of the plant (Viana *et al., 1997*).

Chalcones and dihydrochalcones belong to the class of flavonoids which apart from their antioxidant activity are known for their ability to strengthen capillary walls, thus assisting circulation and helping to prevent and treat bruising, varicose veins, bleeding

gums and nose bleeding (Viana *et al.,* 2003). They may also be useful in the treatment of heavy menstrual bleeding with no apparent cause (Viana *et al.,* 1997). Some flavonoids such as quercetin, rutin, cureumin, silymarin and green tea polyphenols are reputed for their anti-inflammatory effect (Viana et al., 2003). This pharmacological activity may be related to their ability to inhibit cyclooxygenase and lipooxygenase enzymes. Cyclooxygenase and lipooxygenase enzymes act on arachidonic acid metabolism in cell membranes to form potent inflammatory prostaglandins, some of which promote swelling and possibly symptoms as headaches, rashes and joint pains (Zuanazzi, 1999). Flavonoids are a large group of naturally occurring compounds found in fruits, vegetables, grains, bark, roots, stems, flowers, tea and wine (Viana *et al.,* 2003). The flavonoids have received lot of attention because of their impressive antioxidant and anti-inflammatory properties (Lieberman, 2003). A variety of *in vitro* and *in vivo* experiments have shown that selected flavonoids possess anti-allergic, anti-inflammatory, antiviral and antioxidant activities (Holloman *et al.,* 1997, Shoskes *et al.,* 1999).

Quecertin is a natural compound found in plants and it helps to reduce painful swelling because of its potent anti-inflammatory capacities (Shoskes *et al.,* 1999). It is found in fruits, onions and peels of red apples. Quecertin has the same biochemical action as Bestalin® (ubenimex), the potent protein or enzyme inhibitor drug which blocks the entry of viruses like severe acute respiratory syndrome coronavirus into cells (Feng *et al.,* 2001). Quecertin has other remarkable antiviral properties, having been shown to be active against herpes simplex, rotaviruses, paramyxoviruses, polio virus and parainfluenza virus. It does this by inhibiting the enzyme called amino-peptidase N (Feng *et al.,* 2001). Quecertin is an anti-inflammatory, anti-histamine and anti-oxidant agent. It promotes proper circulation of blood in the body (Hertog *et al.,* 1994; Miodini

et al., 1999). Quecertin can be found in fruits (particularly citrus fruits), apples, beans, vegetables, red wine, onions, guava, grape seeds and flavonoid rich extracts (Hollman *et al.,* 1997).

Chalcones isolated from natural products are known to possess several important biological properties, including antifungal (Elsohly *et al.,* 2001; Lopez *et al.,* 2001), leishmanicidal (Chan *et al.,* 2001; Kayser and Kiderlan, 2001), and antimalarial activities (Dominguez *et al.,* 2001; Ram *et al.,* 2001). It has been reported that reactive oxygen and nitrogen species contribute to the pathophysiology of inflammatory conditions and are inhibited by chalcones (Herencia *et al.,* 2001). Hsich *et al.,* (1998) showed that some chalcones present strong inhibitory effect on the release of β–glucuronidase and histamine from rat peritoneal mast cells stimulated with compound 48/80. Other chalcones exhibited potent inhibitory effects on the release of β-glucuronidase and lysosome from rat neutrophils stimulated by an inflammatory agent. Also, arachidonic acid-induced platelet aggregation was inhibited potently by most chalcones (Lin *et al.,* 1997).

Analgesic and anti-inflammatory effects have been observed in flavonoids as well as in tannins (Ahmadiani *et al.,* 1998, 2000; Viana *et al.,* 2003).

2.5. The Role of Plant Secondary Metabolites in Health Maintenance

Over the past years, there has been an exponential growth in the number of reports indicating that excessive free radical production and lipid peroxidation are actively involved in the pathogenesis of a wide number of diseases including atherosclerosis (Chisolm and Steinberg, 2001), cardiac and cerebral ischaemia (Lopez *et al.,* 2001), carcinogenesis (Galati and O'Brain, 2004), diabetic pregnancy (Viana et al., 2003),

DNA damages and digestive disorders (Oyewole, 2003; Bahorun *et al.,* 2005), rheumatic disorders (Hanninen *et al.,* 2000) and they play a major role in aging process. There is overwhelming evidence showing that natural antioxidants play a role in health maintenance and the prevention of chronic degenerative diseases (Bahorun *et al.,* 2005). Futhermore, antioxidation of fats and oils in processed foods may also be prevented by the use of natural oxidation inhibitors or antioxidants (Adegoke and Skura, 1998; Reddy *et al.,* 2005). Consequently, there has been a growing interest in the potential health promoting properties of phytochemicals. Special attention has been given to vitamin C, vitamin E and more particularly to phenolic derivatives, including anthraquinones, xanthones, phenolic acids, phenolic diterpenes, flavonoids, catechins, proanthocyanidins and anthocyanins (Bahorun *et al.,* 2005). These substances have also been reported to exhibit biological effects, including antibacterial, antiviral, anti-inflammatory, antithrombotic, antimutagenic, anticarcinogenic, antidiarrhoeal and antiaging actions (Middleton and Kandaswami, 2002; Brooks, 1998; Caroll *et al.,* 1998; De Bruyne *et al.,* 1999; DiCarlo *et al.,* 1999; Duthie *et al.,* 2000; Middleton *et al.,* 2002; Oyewole, 2003; Viana *et al.,* 2003). The bioactive actions ascribed to polyphenols are almost certainly mediated partly by their free radical scavenging, antioxidant and metal complexing actions (Rice-Evans *et al.,* 1996; Bahorun *et al.,* 2004), their ability to decrease localized oxygen concentration and to decompose peroxides (Aruoma, 2003), their interaction with several enzymes (Chang *et al.,* 1993; DiCarlo *et al.,* 1999) and to synergistic effects with other antioxidants (Filipe *et al.,* 2001). They may prevent tumour development by inducing tumour cell apoptosis by inhibiting DNA topoisomerase II or by causing mitochondrial apoptosis (Galati *et al.,* 2000; Birt *et al.,* 2001; Galati and O'Brian, 2004).

Anthraquinones and chrysophanic acid are very effective inhibitors of skin fungus, mite infestation and microbial diseases. Most members of the *Leguminosae* and *Myrthaceae*

families are rich in these compounds (Anderson *et al.,* 1994; Adebayo *et al.,* 2001; Taylor, 2004; Bahorun *et al.,* 2005). *Cassia alata* anthraquinones were effective inhibitors of *Streptococcus mutans, Staphylococcus aureus, Bacillus subtilis, Micrococcus luteus* and *Pseudomonas putida.* They have also been demonstrated to completely cure bovine skin lesions due to *Dermatophilus congolensis* and mites infestation in rabbits. Chrysophenic acid is also effective in treating psoriasis (Khan *et al.,* 2001, Ali-Emmanuel *et al.,* 2003). Anthraquinones and chrysophanic acid rich plants have been traditionally used to treat eczema, itching and skin infections in humans (Ali-Emmanuel *et al.,* 2003, Bahorun *et al.*, 2005).

Over the past years there has been increasing interest in the use of plant's secondary metabolities in health maintenance. The production of secondary metabolites by plant cell cultures is extensively documented (Zenk *et al.,* 1977; Bahorun *et al.*, 2002; Ramachandra-Rao and Ravishankar, 2002). However, data are limited on the production of secondary compounds in *Psidium guajava* and *Dichrostachys glomerata*. Several investigators have attributed the antidiarrhoeal and anti-inflammatory properties of a number of plants to their flavonoids constituents (Sigh, 1993; Liu, 1995; Oyewole, 2004). Flavonoids (quercetin, rutin, curcumin, silymarin, etc) are reputed for their anti-inflammatory and antidiarrhoeal effects related to their ability to inhibit cyclooxygenase and lipooxygenase enzymes. These enzymes act on arachidonic acid metabolism in cell membrane to form potent inflammatory postaglandins, some of which promote swelling, joint pains and digestive disorders (Capasso *et al.,* 1988; Akah, 1996).

Although traditional medicines help to fill the gaps in modern health care, it is of utmost importance to evaluate the safety and bioefficacy of the extracts used (Bahorun *et al.,* 2005). The phytochemical compositions and biological activities also need to be well

understood (Aruoma *et al.,* 2003; Viana *et al.,* 2003). From the above information, it would appear that despite the massive diversion of interest to synthetic medicine, a significant amount of research has continued in the area of phytomedicine. What has been lacking primarily is the commercial interest to mass produce these phytomedicines and make them available for local and international use. Their use has increased recently in the industrialized world, in part due to disenchantment with conventional medicinal practice (e.g clinical limitations to the management of some chronic diseases) and partly due to a widely held view that herbal medicines are safe. Medicinal plants and herbal medicines are and will continue to be precious natural resource and treasure which can be mobilized for the attainment of health for all in the next few years.

2.6 The Standard Drugs

2.6.1. Acetylcholine (ACh)

Acetylcholine (ACh) (Fig. 2.0) is the endogenous neurotransmitter at cholinergic synapses and neuroeffector junctions in the central and peripheral nervous systems. The actions of ACh are mediated through nicotinic and muscarinic cholinergic receptors, which transduce signals through distinct mechanism (Wang *et al.,* 1995; Singhal and Singhal, 2001). Muscarinic receptors are found primarily on the autonomic effector cells that are innervated by post-ganglionic parasympathetic nerves (Murray *et al.,* 1994). The term parasypathomimetic refers to the effect of the agonists at these sites.

The therapeutic uses of muscarinic receptor antagonists include treatment of gastro-intestinal disorders, specific respiratory conditions, motion sickness, parkinsonian symptoms, and poisoning with cholinesterase inhibitors (Arana *et al.,* 1988). Acetylcholine has effects on both cardiovascular and digestive systems. The effects on the cardiovascular system include: Vasodilation, a decrease in cardiac rate (the negative

chrononotropic effect), a decrease in the rate of conduction in specialized tissues of sinoatrial (SA) and atrivoentricular (AV) nodes (the negative dromotropic effect), and a decrease in the force of cardiac contraction (the negative inotropic effect) (Nazarine *et al.,* 1998).

On the gastrointestinal system, ACh produces increase in tone, amplitude of contractions and peristaltic activity of the stomach and intestines, as well as enhanced secretary activity of the gastro-intestinal tract. The enhanced motility they may be accompanied by nausea, belching, vomiting, intestinal cramps and defaecation (Malone *et al.,* 1999; Eno *et al.,* 1999). The anticholinesterase agents prolong the existence of Ach after it is released from cholinergic nerve endings. Tubocurarine is an antagonist of ACh that acts at nicotinic receptors. It is a competitive non-depolarising agent (Futter *et al.,* 1983). Neostigmine is an anticholinesterase agent which prolongs the action of ACh. The function of acetycholinesterase (AChE) is to terminate the action of ACh at the junctions of various cholinergic nerve endings with their effector organs in post-synaptic sites (Futter *et al.,* 1983; Taylor and Radic, 1994).

Drugs that inhibit AChE are called anticholinesterase (anti-ChE) agents. They cause ACh to accumulate at cholinergic receptor sites and, thus, are potentially capable of producing effects equivalent to excessive stimulation of cholinergic receptors throughout the central and peripheral nervous systems (Taylor and Radic, 1994; Dzenda, 2004). The muscarinic action of ACh is blocked selectively by atropine through competitive occupation of cholinergic receptors sites on the autonomic effector cells and on the muscarinic receptors of autonomic ganglion cells (Uchendu and Leck, 1999a, b). Some cholinergic inhibitors have been isolated from some plants ethnomedically used for diarrhorea treatment (Choudhury and Haruna, 1994; Haruna and Choudhury, 1997).

According to Haruna and Choudhury (1997), the aqueous extract of *Aristolochia albida* Duch exhibited significant relaxation of the spontaneous pendular contraction of isolated rabbit duodenum. The extract at moderate doses abolished ACh-induced contractions on isolated rabbit duodeum, thus mimicking atropine.

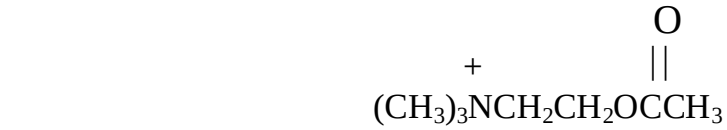


Fig 2.0: Chemical Structure of Acetylcholine

Since muscarine was characterized originally as acting relatively and selectively at autonomic effector cells to produce qualitatively the same effects as ACh, actions of ACh and related drugs at these sites are referred to as muscarinic. All of the actions of ACh and its congener at the muscarinic receptors can be blocked by atropine (Pesic and Grbovic, 1999). The nicotinic actions of cholinergic agonists refer to their initial stimulation of autonomic ganglion cells and the neuromuscular junction, and such actions are comparable to those of nicotine (Walker *et al.*, 2001).

2.6.2. Atropine

According to Martinotti *et al* (1997), atropine (Fig. 2.1) is the best known muscarinic receptor antagonist. Atropine and related compounds are competitive antagonists of the actions of ACh and other muscarinic agonists; they compete with such agonists for common binding site on the muscarinic receptor. At the neuromuscular junction, where the receptors are principally or exclusively nicotinic, extremely high doses of atropine or related drugs are required to cause any degree of blockade (Menezes, 1998). Muscarinic receptor antagonists prevent the effects of ACh by blocking its binding to muscarinic cholinergic receptors at neuroeffector sites on smooth muscles, cardiac muscle, and gland cells, in peripheral ganglia; and in the central nervous system (Aguiyi *et al.*, 1997).

Some plant extracts have been shown to possess muscarinic receptor antagonist property (Haruna and Choudhury, 1997; Shamsa, 1999; Abdullahi *et al.*, 2001a, b).

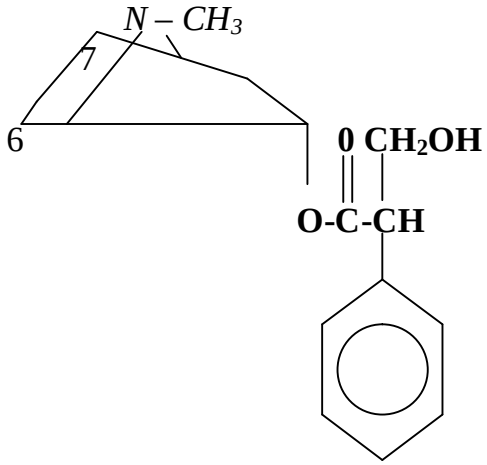


Fig. 2.1: Chemical Structure of Atropine

2.6.3 Histamine

The name histamine (Fig. 2.2) is derived from Greek word for tissue, *Histos.* Best *et al* (1927) isolated histamine from very fresh samples of liver and lungs, thereby establishing that this amine is a natural constituent of the body. It is now evident that endogenous histamine plays a role in the allergic responses and it is an important regulator of gastric acid secretion (Lichtenstein *et al.,* 1994). Almost all mammalian tissues contain histamine in amounts ranging from less than 1 to more than 100μg/g.

The mast cell is the predominant storage site for histamine in most tissues. The concentration of histamine is particularly high in tissues that contain the bronchial tree, and the intestinal mucosa (Lagunoff *et al.,* 1983).

There are at least three distinct classes of receptor for histamine, designated H_1 (Ash and Schild, 1966), H_2 (Black *et al.,* 1972) and H_3 (Arrang *et al.,* 1983). Histamine stimulates, or more rarely relaxes, various smooth muscles. Contraction is due to the activation of H_1 receptors and relaxation for most part due to activation of H_2 receptors (Phadnis *et al.,* 1995). The classical effect of histamine on the intestinal muscle is contraction (Yoon *et al.,* 1994). H_1 receptors are coupled to phospholipase C, and their activation leads to formation of inostol – 1, 4, 5 triphosphate (IP_3) and diacylglycerol from phospholipids in the cell membrane, IP_3 causes a rapid release of Ca^{2+} from the endoplasmic reticulum (Dickenson and Hill, 1993). Diacylglycerols and Ca^{2+} activate Ca^{2+}/calmodulin – dependent protein kinases and phospholipase in the large cell to generate the characteristic response (Shamsa *et al.,* 1999). Some plant extracts are reported to contain histamine receptor inhibitors (Choudhury and Haruna, 1994; Haruna and Choudhury, 1997). Antihistaminic agents such as mepyramine are known to inhibit the actions of histamine at the H_1 and/or H_2 receptor sites (Gomes *et al.,* 1994; Nkeh *et al.,* 1996).

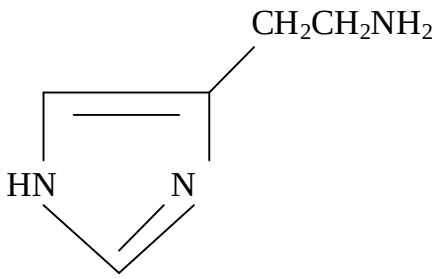


Fig. 2.2: Chemical Structure of Histamine

The bronchoconstriction and contraction of the gut are mediated by H_1 receptors which are blocked by classical antihistamines (now more properly described as H_1 – receptor antagonists) (Ash and Schild, 1966). Other effects, most notably gastric secretion, are the result of activation of H_2 – receptors and, accordingly, can be inhibited by H_2 – receptor antagonists. Some responses, such as the hypotension that results from vascular dilation, are mediated by both H_1 and H_2 receptors (Black *et al.,* 1972; Nkeh *et al.,* 1996).

2.6.4. Imodium

Imodium® (Loperamide hydrochloride) is indicated for the symptomatic control of acute and chronic diarrhoea. In patients with an ileostomy, it can be used to reduce the number and volume of faeces, and to harden their consistency. Loperamide binds to the opiate receptors in the gut wall.

Consequently, it inhibits the release of acetylcholine and postaglandins, thereby reducing propulsive peristalsis and increasing intestinal transit time (Karim and Adaikan, 1977). Loperamide increases the tone of the anal sphincter, thereby reducing incontinence and urgency. Studies on distribution in rats show high affinity for the gut wall with a preference for binding to receptors of the longitudinal muscle layer. Excretion mainly occurs through the faeces. The therapeutic effect of Loperamide is believed to be due to its antimotility and antisecretory properties (Couper, 1987; Abdullahi *et al.,* 2001a, b). The capsule contains titanium dioxide yellow ferric oxide, indigotindisunphonate sodium, gelatin, black ferrous oxide, erythrosine sodium. Also included are lactose, maize starch, talc, and magnesium stearate (Janssen, 2001). Flavonoids, which are abundant in some plants, have been demonstrated to inhibit contractions induced by spasmogens (Macander, 1996, Capasso *et al.,* 1988), inhibit intestinal secretion and intestinal transit in mice comparable to loperamide (Dicarlo *et al.,* 1993; Abdullahi *et al.,* 2001a, b).

2.7.0 **Taxonomy of *Dichrostachys glomerata* (Forsskal)**

Kingdom	:	*Plantae, Planta, Plantse*
Division	:	*Spermatophytha*
Sub-division	:	*Angiospermae (Angiosperms)*
Class	:	*Dicotyledonea (Dicotyledons)*
Order	:	*Leguminales*
Family	:	*Leguminosae*
Sub-family	:	*Mimosoidae*
Genus	:	*Dichrostachys*
Species	:	*glomerata*
Author	:	*Forsskal*
Determiner	:	*Mallam Umar Shehu Gallah*

2.7.1 Synonyms

- *Dichrostachys cinerea (Linnaeus)*
- *Dichrostachys nutans (Pers.) Benth*
- *Dichrostachys platycarpa (Welw.)*
- *Mimosa glomerata (Forssk.)*
- *Mimosa cinerea (Linnaeus)*
- *Cailliea dichrostacys (Gull & Perrott.)*



Plate 2.0: ***Dichrostachys glomerata*** **Forsskal Plant and Flowers.**

2.7.3 Common name(s)

English: Christmas tree, sickle bush, aroma, Kalahari Christmas tree, Chinese lantern tree, marabou-thorn, marabou, sen Domeng, Saint Domingue, acacia Saint Domingue. Del marabua (Cuba), Kalahar – Weihnachstsbaum (German), Kakada (the Sudan).

2.7.4 Local name(s)

Hausa: Dundu. Fulani: Burli. Yoruba: Kara. Igbo: Ami ogwu

2.7.5 Geographical range

Native range: Native to Africa.

Known introduced range: *Dichrostachys glomerata* (Plate 2.0) has been introduced to the West Indies during the 19th century mainly to Cuba, Hispaniola, Guadeloupe, Marie-Galante, and Martinique. It invades fields, wastelands, road sides, and other disturbed areas. In Cuba, the trees grow so dense that the branches meeting at five to six meters above the ground cause the roads and paths cut through to appear as tunnels (Kudela, 1978; Cowan, 1998).

2.7.6 Habitat

Gregarious, very invasive in fallows and difficult to eliminate because of its active suckering, liable to produce dense thickets. The distribution of the tree is probably effected in part by cattle carrying the seeds in the hooves, when driven or transported over the grazing land. Cattle also greatly relish the seeds and it is possible that they are capable of germination after passing through the animal (Weir, 1927; Cowan, 1998).

2.7.7. Description of *D. glomerata*

Thorny shrub or small tree reaching 3-7m in height. Bark on trees thick and vertically fissured, very fibrous, slash cream-coloured to light yellow. Strong (8-10cm), slightly

recurved alternate woody thorns growing at straight angle from the branches, bearing leaves at their base. The leaves are acacia-like, bipinnate, 4-8 cm long, with 5-15 pairs of pinnae having 12-30 pairs of leaflets each. The leaflets measure 6-8mm long x 2.5mm wide. Very characteristic bicoloured flowers, on petiolated, cylindrical, dense pendulous spikes, bottle brush like, 6-8cm long (Plate 2.0), fragrant, flowering between February and May. The upper flowers in the spikes are sterile and reddish-purple in colour, with protruding staminodes, the lower flowers are fertile and pale yellow to cream in colour. Pods are brownish or yellowish in colour, about 10cm long, indehiscent, twisting to form strangely shaped bundles that remain on the tree for a long period, usually four-seeded; 34,000 clean seeds per kilogram (Almeida, 1974; Kudela, 1978; Cowan, 1998 Tassin, 1999).

2.7.8. Products and Uses

The pods form a valuable source of protein in Central Tanzania, when they ripen and fall in August, a time when grazing is scarce. The leaves, twigs and pods are browsed by stock and the flowers appreciated by bees. The tree is considered to be a valuable firewood and charcoal species, notably in Dodoma in Central Tanzania. The wood is very heavy and hard (fine dark brown heart wood), termite resistant, but usually of small dimensions. It is used in the construction of houses, for tool handles, and as support to local grapes and yams. The root-bark yields a strong fibre like that of some acacias, a fibre called *medi* in Hausa and *udo* in Ibo used in making closely-plaited bags and other articles. The roots themselves, after removal of the bark, are used in Ghana to twist into baskets (Weir, 1927; Dalziel, 1937; Cowan, 1998; Harborne, 1998).

2.7.9. Ethnomedical Uses

In Nigeria, the bark is used to treat dysentery and diarrhoea, tooth aches and for elephantiasis (Dalziel, 1937). The leaves are used to treat gonorrhoea and boils. It is also a remedy for stomach problems and can remove poisons from snake-bites (Dalziel, 1937, Harborne, 1998). In Sierra Leone, the bark seems to have a special reputation as a vermifuge. It is scraped pounded and macerated in cold water with spices and lime juice added. The mixture is given both for dysentery and diarrhoea and for worms (Harborne, 1998). A simple decoction or a cold infusion of the macerated inner bark is given as a stomachic and vermifuge, and the same is taken internally as a remedy for urethral discharges. For elephantiasis, the above mixture is taken internally, and the pounded bark soaked in hot water and applied locally. In Liberia, a decoction of the bark is used, after cooling, as a wash to relieve the inflammation of gonorrhoea and after circumcision (Harborne, 1998). In Senegal and Guinea, the root taken in milk is regarded as diuretic and slightly laxative. An infusion of the root is given to woman after childbirth, and put in the food of young children at weaning as a remedy for stomach problems (Dalziel, 1937). In Tanzania, a decoction of the root bark is used internally as a remedy for diarrhoea and externally for foot-and-mouth disease (FMD) in cattle (Harborne, 1998). In Liberia, an anklet of the bark is a charm against snake-bite, or is tied to prevent spread of the venom (Dalziel, 1937; Harborne, 1998). In Zimbabwe, the dried leaf and root are smoked for pulmonary tuberculosis and chest ailments and for nasal congestion. The leaf is also chewed to ease colic and heartburn and can be made into tea to remedy stomach ailments and diarrhoea (Cowan, 1998). Dried powdered bark is directly applied to skin eruptions, sores, blisters and abscesses for both man and animals (Harborne, 1998). In South Africa, a lotion made from the leaves and bark is used as a wound cleaner and healer. Dried powdered bark is sprinkled on wounds to promote healing (Harborne, 1998; Tassin, 1999). Snake-bites, scorpion stings and bee stings are also

treated with the leaf and bark – the leaf is chewed well and then applied and bound over the area.

The Pedi and Lobedu tribes use the pod as anti-witchcraft charm. Sickle bush is believed to ward off evil if it grows near a residential area. It is also used to treat pneumonia, epilepsy, internal ulcers and dysentery (Harborne, 1998).

2.8.0. Taxonomy of *Psidium guajava* (Linnaeus).

Kingdom	:	*Plantae, Planta, Plantes,* Vegetal
Division	:	*Spermatophytha*
Sub-division	:	*Angiospermae*
Class	:	*Dicotyledonea (Dicotyledones)*
Order	:	*Myrtales*
Family	:	*Myrtaceae*
Genus	:	*Psidium*
Species	:	*guajava*
Author	:	Linneaus (Linn.)
Determiner	:	Mallam Umar Shehu Gallah

2.8.1. Synonyms

Psidium pumilum (Vahl) Griseb.



Plate 2.1: ***Psidium guajava*** **Linnaeus Plant.**

2.8.2. Common names:

Guava (English), *goiaba* (Brazil), *guayaba* (Spanish), *goavier* (French), *djambu, gouayave, goyave, petokal, tokal, banjiro, guyave, guave, kuawa, abas, jambu botu, pichi, posh, enandi* (Taylor, 2004; Rain-tree, 2005).

2.8.3. Description

Guava, also known as guayaba, goiaba and goavier, is an evergreen shrub or small tree 2 to 8m in height and up to 40cm in diameter at breasts height (Morton, 1987; Tona *et al.*, 1999). The plant is characterized by smooth, dun (copper) coloured bark between thin irregular scales that peel off. Guava bark is between 5 to 8mm thick. The sapwood is light brown and the heartwood is reddish brown, hard, heavy and strong (Little and Wadsworth, 1964). The guava tree possesses elliptical, apposed leaves, white flowers and pear-shaped fruit with a prominent, persistent calyx (Plate 2.1). They are at first hard and green, becoming softer, and yellow at maturity and 3 to 5cm long. Inside the thin rind is a sweet-tart flavoured pink or yellow pulp containing many hard, angular, yellow seeds about 2mm long (Stevens *et al.,* 2001).

2.8.4. Geographical Range

Guava originated somewhere in the Neotropics and was spread nearly to the extent of its adapted climate before the arrival of Europeans (Morton, 1987). Seeds found in Peruvian archeological sites seem to indicate that it originated in that area (Rain-tree, 2005). Guava tree is now cultivated throughout the tropical and sub-tropical regions of the world (Howard, 1989; Taylor, 2004).

2.8.5. Tribal and Herbal Medicinal Uses

Guava may have been domesticated in Peru several thousand years ago. An infusion or decoction made from the leaves and/or bark has been used by many tribes for diarrhoea and dysentery throughout the Amazon, and Indians also employ it for sore throats,

vomiting, stomach upsets and also to regulate menstrual periods (Tona *et al.,* 1999; Rain-tree, 2005). A decoction of the bark and/or leaves or a flower infusion is used topically for wounds, ulcers and skin sores. Flowers are also mashed and applied to painful eye conditions such as conjunctivitis or eye injuries (Rain-tree, 2005). Guava leaves are in the Dutch Pharmacopoeia for the treatment of diarrhoea, and the leaves are still used in traditional medicine for treatment of diarrhoea in Latin America, Central and West Africa (Taylor, 2004). Worldwide uses of this plant are shown in Table 2.0.

2.8.6. Phytochemicals and Biological Activities

Human clinical trials with guava extracts indicate effectiveness in treating diarrhoea in adults. Guava leaf extracts and fruit juice have been clinically studied for infantile diarrhoea. In a clinical study with 62 infants with infertile rotaviral enteritis, the recovery rate was 3 days (87.1%) in those treated with guava, and diarrhoea ceased in a shorter time period than the control (Taylor, 2004; Rain-tree, 2005).

Guava has many different properties that contribute to its antidiarrhoeal effect: It has been documented to have pronounced antibacterial, antiamoebic, and antispasmodic activity. It has also been shown to have a tranquilizing effect on intestinal smooth muscles. It inhibits chemical processes found in diarrhoea and aid in reabsorption of water in the intestines (Deluchi *et al.,* 1998). Alcoholic leaf extract was reported to have a morphine-like effect by inhibiting the gastric intestinal release of chemicals in acute diarrhoea. This morphine-like effect was thought to be related to the chemical quercitin (Deluchi *et al.,* 1998). In addition, lectin chemicals in guava were shown to bind to *Escherichia coli* (a common diarrhoea causing-organism), preventing its adhesion to the intestinal wall. Thus, it prevents infection and the diarrhoea that may occur thereafter (Deluchi *et al.,* 1998; Lozaya,, 2002).

The bark and leaf extracts also have shown *in vitro* efficacy against some diarrhoea-causing bacteria such as *Staphylococcus, Shigella*, *Salmonella, Bacillus, Clostridium* and *Pseudomonas*. It has also antifungal, antiyeast (*Candida),* antiamoebic and antimalarial actions (Tona *et al.,* 1999; Stevens *et al.,* 2001).

Guava is rich in tannins, phenols, triterpenes, flavonoids, saponins, carotinoids, lectins, vitamins, fibre and fatty acids. Guava fruit is higher in vitamin C than citrus (80mg of vitamin C in 100g of fruit) and contains appreciable amount of vitamin A as well. Guava's main phytochemicals include alanine, ascorbic acid, D-galactose, flavonoids, gallic acid, quecertin, tannins, terpenes and urolic acid (Stevens *et al.,* 2001; Taylor, 2004).

Table 2.0: **World wide Uses of *Psidium guajava* leaves and root-bark**

Country	Ethnomedical Uses
Amazoni	Diarrhoea, dysentery, menstrual disorders, stomach ache.
Brazil	Anorexia, catarrh, cholera, diarrhoea, dysentery, gastric insufficiency, sore-throat, ulcerative vaginal discharge.
Cuba	Cold, dysentery, diarrhoea.
Ghana	Astringent, cough, diarrhoea, dysentery, tooth ache.
Haiti	Antiseptic, astringent, dysentery, diarrhoea, epilepsy, itch, pile, scabies, sore-throat, stomach ache, wounds.
Indian	Anorexia, cerebral ailments, childbirth, convulsions, epilepsy, nephritis.
Malaya	Dermatosis, diarrhoea, epilepsy, menstrual stimulant.
Mexico	Deafness, diarrhoea, scabies, itch, stomachache, swelling ulcer, worms, wounds.
Peru	Conjunctivitis, cough, gout, diarrhoea, digestive aid, dysentery, oedema, vaginal discharge, worms, pneumonia, vomiting.
Phillippines	Astringent, sore, wound
Trinidad	Antibacterial, astringent, diarrhoea, dysentery
Elsewhere	Anorexia, antibacterial, boils, bowel disorders, bronchitis, catarrh, colds, colic, convulsion, cough, diarrhoea, dysentery, oedema, epilepsy, fever, gingivitis, haemorrhoids, jaundice, menstrual promoter, nausea, nephritis, sore-throat, stomach problems, swelling, toothache, ulcer, worms, wounds.

Sources: (Lutterodt, 1989; Tona *et al.*, 1999; Taylor, 2004 Rain-tree, 2005).

2.9.0. Diarrhoea

Diarrhoea may be defined as a condition in which an adult's daily stool exceeds 200g and contains 60-95% water (Abdullahi *et al.*, 2001a, b). It is also defined as the passage of watery stools usually at least three times in a 24 hour period (Weber, 1976), the passage of abnormally soft, watery faeces or the passage of faeces at unusually frequent intervals (Hassan and Hassan, 2004). However, it is the consistency of the faeces rather than the number that is most important. Frequent passing of formed faeces is not diarrhoea. Babies fed only breast milk often pass loose "pasty" stools, this is not diarrhoea (Abdullahi *et al.*, 2001a, b).

Diarrhoea is an intestinal disorder characterized by abnormal fluidity and frequency of faecal evacuations. Generally, the result of increased motility in the colon may be an important symptom of such underlying disorders as amoebic dysentery, lactose intolerance, gastrointestinal tumour, colibacillosis and inflammatory bowel disease (Weber, 1976). Diarrhoea could result from inflammatory condition, infections, helminth infestation, malabsorption, fear and anxiety, irritation of bowel and poisoning (Hassan and Hassan, 2004). Acute diarrhoea is a common life-threatening condition of calves, foals, kids, lambs and children. The rapid clinical progression of the condition demands that early and aggressive fluid and electrolyte therapy is instituted in addition to chemotherapy targeting the underlying cause (Carlson, 1989; 1999; Ecke *et al.*, 1998). When fluid and electrolyte replacement strategies in horses are reviewed, the intravenous route is most frequently described. However, oral rehydration solution (ORS) provides a simple, cheap, and readily available means of replacing substantial quantities of fluid and electrolytes in animals (Grove-White, 1997).

The therapeutic targets in the treatment of diarrhoeic animals and man include the correction of hypovolaemia and metabolic acidosis (Michell *et al.,* 1992; Grove-white, 1997), the maintenance of body condition and assisting in the repair of the gut mucosa (Brooks, 1998). Diarrhoea is common in the tropics. Indeed, in certain parts of the developing world diarrhoea produces more illness and causes death of more infants and children than all other diseases combined (Weber, 1976; Foldenauer *et al.,* 1998). It causes morbidity and mortality in livestock inflicting significant economic losses (Akah, 1996). The growth of the poultry industry in Nigeria has been hampered by several factors including diseases (Adene, 1997). Colibacillosis has been identified as a significant disease of poultry in Nigeria (Kwaga *et al.,* 2004). Some serotypes of *Escherichia coli* cause diarrhoea in piglets, resulting in great economic losses to the pig industry world-wide (Aning, 1986).

During diarrhoea there is an increased loss of water and electrolytes (sodium, potassium, calcium, chloride and bicarbonate) in watery faeces. Dehydration occurs when these losses are not replaced adequately, and a deficit of water and electrolytes develops. Clinical signs such as increased thirst, restlessness or irritable behaviour, decreased skin turgor, dry mucous membranes, sunken eyes, etc, are associated with dehydration. Hypocalcaemia has been reported in foals in association with previous episodes of diarrhoea (Beyer *et al.,* 1997), and in children in association with rotavirus diarrhoea (Foldenauer *et al.,* 1998), and dehydration (Menezes *et al.,* 1998), some of the dehydrated infants developed tetanic convulsions (Grove-White and Mitchell, 2001).

The antidiarrhoeal drugs include products such as absorbents (e.g. Kaolin, attapulgate, smectate, activated charcoal, cholestryamine, etc). These agents are promoted for the treatment of diarrhoea on the basis of their ability to bind and inactivate bacteria toxins

or other substances that cause diarrhoea, and their ability to protect the intestinal mucosa (Aning, 1986). The antimotility drugs (e.g. loperamide hydrochloride, diphenoxylate with atropine, codeine, etc) are employed to reduce the frequency of faecal passage. However, they can cause severe paralytic ileus in children. Sedation may occur at usual therapeutic doses and fatal central nervous system (CNS) toxicity has been reported (Ahmad *et al.,* 1998). Products such as Ivax veterinary® combine the properties of absorbent, antimicrobial and antimotility actions. However, such combinations are irrational and their cost and side effects are substantially higher than for individual drugs (Ahmad *et al.,* 1998).

In recent years multiple drug resistance in human, animal and plant pathogenic microorganisms have developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases (Davis, 1988; Lopez *et al.,* 2001). In addition to these problems synthetic antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions (Ahmad *et al.,* 1998). Therefore, there is an urgent need to develop alternative antidiarrhoeal and antimicrobial drugs from medicinal plants for the treatment of infection and infestation diseases (Clark, 1996; Cordell, 2000; Gulliice *et al.,* 2004; Bahorun *et al.,* 2005).

Diarrhoea ranges from mild and socially inconvenient illness to a major cause of malnutrition among children of developing countries and the cause of 4-5million deaths throughout the world annually (Abdullahi et al., 2001a, b). To combat the problems of diarrhoea in developing countries, the World Health Organisation (WHO) has constituted a Diarrhoeal Disease Control programme (DDC) which includes studies of traditional medicinal practices, together with the evaluation of health education and

prevention approaches (Mitchell, 1983; Abdullahi *et al.*, 2001a, b). Efforts are being made in different countries to carry out research into traditional medicine and to integrate it with modern orthodox medicine.

In conclusion, information from the Literature review indicates that extracts from *Psidium guajava* and *Dichrostachys glomerata* are employed for diarrhoea and dysentery treatment, in man and his animals.

Mammals such as goats, cattle, sheep and horses browse the leaves, flowers, and fruits of these plants probably for self medication. It is most likely that many plant foods of animals contain secondary compounds that were inhibitory agents to pathogens, thus, aiding to preserve health of the ingestor. It is equally likely that humans watching the behaviour of sick animals discovered the medicinal properties of many plants early on in our history. Whereas the use of *P. guajava* as antidiarrhoeal agent have been studied in China, India and Mexico, the use of *D. glomerata* as antidiarrhoeal agent has not been investigated to the best of my knowledge. From the various ethnomedical uses of these plants as antidiarrhoeal agent, it is very likely they possess definite antimotility, anti-secretory, inflammatory and antibacterial activities which need to be investigated. Since the contractile receptors in the intestines have been identified to include those of Acetylcholine and histamine, it is important to use these agonists and their antagonists in order to establish the probable mode of action of these plants – *P. guajava* and *D. glomerata.* Also, since loperamide was reported to antagonize diarrhoea induced by castor oil, it is important to use these standard antidiarrhoea agents in order to establish the modes of action of these plants. This may be an important step in laying a scientific basis for the use of *P. guajava* and *D. glomerata* as antidiarrhoeal agents.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.2 Apparatus

Animal cages and drinkers (for mice, rats, guinea pigs and rabbits), Petri dishes, 5.0mm cork borer, curved 19G needles with blunt end, universals bottles, porcelain dishes, thimbles, weighing balance (Avery, Birmingham) beakers, electric oven, autoclave, recording microdynanometer 7050 (Ugo Basile, Milan – Italy), air pump (Gast, Benton Harbor, Michigan – USA), hot water bath (Gallenhamp, England), thermostat (Churchill, thermocirculator, Walmgate Road, Middx-England), sensitive chemical balance-(Mettler P162, CH8606 Grefensec-Zurich, Switzerland), syringes and needles, conical flasks, glass rods, test tubes, reagent bottles, thermostatically controlled isolated organ/tissue bath (25ml SRI), water deionizer (Permutit 500), transducer, 1 litre reservoir for physiological solution (Pyrex, England), spiral condenser, oxygenated tubes, weight (tension) 1g, transparent plastic rule, cotton wool, cotton thread, table clock, tissue clamp, strile cork borer, strile Petri-disches.

3.3. Chemicals, Test Substances and Physiological Salt Solution

The chemicals used in this study include ethanol (Analar®, BDH, England), formalin and chloroform (Analar® BDH, England). Test substances included the ethanolic extract of *Psidium guajava* and *Dichrostachys glomerata* (locally collected in Vom, Jos South Local Government Area, Plateau State), acetylcholine hydrochloride (Sigma, USA), histamine hydrochloride (Sigma, USA), mepyramine sulphate (Bayer, Germany), Imodium® (Janssen, Pharmaceutical, Belgium). The other substances used included activated charcoal (Merck), castor oil (Tina Pharmaceutical, Nigeria), ampicillin (Fidson Health Care, Nigeria), indomethacin, tragacanth (locally collected from *Astragalus*

gumnifera Labil) and fresh egg Albumen. The physiological salt solution used for the *in vitro* tissue studies was Tyrode`s solution with the following composition in gram per litre of deionized water: NaCl, 8.0; KCl, 0.2; MgCl, 0.1; $CaCl_2$, 0.2; NaH_2 $PO_4.2H_2O$, 0.05; $NaHCO_3$, 1.0; Glucose, 1.0 and aerating gas.

3.4. Animals

Swiss albino mice (*Mus musculus*) and Wistar rats (*Rattus norvagicus*) were purchased from the Experimental Animal Unit, National Veterinary Research Institute, Vom. They were kept in mice and rat cages, respectively, in the Physiology and Pharmacology Laboratory of the Department of Veterinary Physiology and Pharmacology, ABU, Zaria until use. The mice and rats were fed on commercially formulated chicken growers mash (Guinea Feed Ltd, Sapele), maize bran (dussa) and groundnut cake mixed in a ratio of 4:2:1 made into pellets with wheat flour used as binder. Water was provided *ad labium.* The mice and rats were allowed to acclimatize for 14 days prior to their use in the experiment.

Domestic rabbits (*Orgatolagus cunniculi*) used in the study were purchased from Animal Science Department, ABU, Zaria while the guinea pigs (local breed) were purchased from Samaru Markets, Zaria. The rabbits and guinea pigs were housed in animals' pens in rabbit and guinea pig cages, respectively, in the Faculty of Pharmaceutical Sciences, ABU, Zaria, under satisfactory husbandry conditions until used. They were fed with chicken growers mash (Guinea Feed Ltd; Sapele) combined with cabbage or *Amaranthus species* (green vegetable). Water was provided *ad libitum*.

3.4.1. Study Animals

Forty, 6 – 25 months old goats of the red Sokoto breed from the Livestock Investigation Department, NVRI, Vom that weighed between 8 – 20kg were used. The goats had

naturally acquired mixed parasitic infections with gastro-intestinal nematodes (*Haemonchus contortus, Trichostronglylus sp*, and *Oesophagostomum sp.*). Infections were confirmed at Parasitology Department, NVRI, Vom, before the beginning of the study by collecting faecal samples from the animals per rectum and the number of nematodes egg therein determined by the McMaster technique with a sensitivity of 50 eggs per gram of faeces (epg) (Hansen and Perry, 1994; Ademola *et al.,* 2004). Only those animals whose egg counts exceeded 750 epg were used. Before the experiment, the animals were washed with Taktic® Amitraz (Intervet International, The Netherland) to rid them of ticks. The goats were fed on fresh grass, hay, concentrate and water *ad libitum*.

3.5. Test Organisms

Bacteria used in this study were pure isolates obtained from stock cultures in Bacteriology Department, National Veterinary Research Institute Vom. They included *Staphylococcus aureus, Escherichia coli* and *Salmonella typhi*. The identity of the bacteria was confirmed by E.S.O. Ekundayo (a Laboratory Scientist) formerly of the FCVMLT, NVRI, Vom) to be those of *Staphylococcus aureus, Escherichia coli* and *Salmonella typhi.*

3.6. Methods

3.6.1. Collection, Drying and Grinding of *P. guajava* and *D. glomerata* leaves

Fresh leaves of *Psidium guajava* and *Dichrostachys glomerata,* respectively, identified by Mallam M. Abdul of the Savannah Herbarium, NAPRI, Shika Zaria and authenticated by Mallam Umar Shehu Gallah (a taxonomist) in the Department of Biological Science, ABU, Zaria was carefully collected from Vom in Vwang district of Jos South L.G.A; Plateau State, Nigeria. The plant materials were collected with the aid of Mallam Gambo Kano and M. Maigari (herdsman) in Livestock Investigation Department, NVRI, Vom. Voucher specimen “Okpara No. 1 and 2 has been preserved for reference in both NAPRI

and Biological Science Herbarium.The fresh leaves were air dried for 4 weeks in the Physiology and Pharmacology Laboratory of the Department of Veterinary Physiology and Pharmacology ABU, Zaria, between March and April, 2005 (Plates 3.0; 3.2). The dried leaves were grounded using mortar and pistle and carefully packed in clean leather bags (Plates 3.1; 3.3), weighed and stored under room temperature until use.

3.6.2. Preparation of Crude Ethanolic Extract from the powdered leaves of *P. guajava* and *D. glomerata*

The extraction procedure is as described by Harborne (1998). Two hundred grams (200g) of the powdered leaf material of *Psiduium guajava* were packed into a thimble and transferred into Soxhlet extractor (Plate 3.4). The extraction was carried on with 1 Litre of 95% ethanol until there was no more colour change in the ethanol, indicating that the extraction was complete. That took about 72 hours to complete, the ethanolic extract harvested was then concentrated *in vacuo* using a rotary evaporator which separates the ethanol from the extract. The ethanol remaining in the extract was removed by placing small volumes in porcelain dishes in the oven set at 80^0C until the weight remained constant. The extract was then collected in a plastic container, closed tightly (air and water tight) and stored in refrigerator at 4^0C until required.

3.6.3 Acute Oral Toxicity Studies of Crude Ethanolic Extract of *P. guajava* and *D. glomerata* Leaves in Mice

Acute oral toxicity studies of the ethanolic leaf extract of *P. guajava* and *D. glomerata* was carried out using modified Locke's method (Locke, 1983). A suitable solvent for the extracts used in the studies were determined by trying to dissolve the extracts using three different solvents (distilled water, groundnut oil and propylene glycol).

The maximum convenient concentration (MCC), which was the maximum amount of extract dissolved in 1.0ml of distilled water such that the solution was delivered through a 19G needle at room temperature, was determined by measuring 1.0g of the extract (*P. guajava* and *D. glomerata*, respectively) and adding 0.05ml of distilled water at a time, until the solution could be easily delivered through the needle. From the volume of solvent used, the MCC of the extract in g/ml was determined and a stock solution was made for use. The maximum convenient volume (MCV) that can be administered to mice by oral route is 5ml (Ibrahim, 1984). Therefore, the maximum tolerated dose (MTD), g/kg of the extract used in the studies was calculated by multiplying the MCC (g/l) by the MCV (m/kg) as described by Ibrahim (1984).

In these toxicity studies, the mice were fasted for 24 hours and deprived of water 12 hours before the administration of the extracts. An insulin syringe fitted to a curved 19 – gauge needle with blunt end was used to administer the extract orally. All the mice were weighed and identified with marks using indelible marker pen. Clinical signs of toxicity in mice were observed and recorded. The studies were carried out in two stages. The first stage was the pilot acute toxicity studies and the second stage was that of median lethal dose (LD_{50}) determination.

3.6.4. Pilot Acute Toxicity Studies of *P. guajava*

Fifteen mice (18 – 25.4g) of both sexes, arranged in five groups (1 – 5) of three mice each randomly, were used to determine the amount of extract of *P. guajava* that was capable of bringing about 0 – 100% death when given orally to mice.

Group 1 received 10,000mg of the extract per kg body weight.

Group 2 received 1,000mg of the extract per kg body weight.

Group 3 received 100mg of the extract per kg body weight

Group 4 received 10mg of the extract per kg body weight

Group 5 served as control and was given only the vehicle, distilled water at 5ml per kg body weight.

3.6.5. Determination of LD_{50} of Crude Ethanolic Extract of *P. guajava* Leaf in Mice

From the pilot study the range of doses for LD_{50} was selected to be between 1000mg to 10,000mg per kg body weight of mice. Thereafter, twelve mice were arranged in 4 groups of 3 Mice each as follow:

Group 1 received 4,000mg of the extract per kg body weight of mice.

Group 2 received 3,000mg of the extract per kg body weight of mice

Group 3 received 2,000mg of the extract per kg body weight of mice

Group 4 received 1,000mg of the extract per kg body weight of mice

From the data collected from the above study the range of doses for LD_{50} was selected to be between 1,000mg to 2000mg per kg body weight of mice twelve mice were further treated in four groups of three mice each.

Group 1 received 1000mg of extract per kg body weight *per os*

Group 2 received 1300mg of extract per kg body weight *per os*

Group 3 received 1,600mg of extract per kg body weight *per os*

Group 4 received 1,900mg of extract per kg body weight *per os.*

From the data collected in the above studies, the median lethal dose (LD_{50}) of *Psidium guajava* extract in mice was calculated by taking the geometric mean between the lowest lethal dose that produced 100% death and the highest non–lethal dose.

3.6.6. Determination of LD_{50} of Crude Ethanolic Extract of *D. glomerata* Leaf in Mice

From the pilot studies, there range of doses for LD_{50} was selected to be between 1,000mg and 4,000 per kg body weight of mice. Thereafter twelve mice were further treated in four groups of three mice each

Group 1 received 4,000mg of the extract per kg body weight *per os*

Group 2 received 3,000mg of the extract per kg body weight *per os*

Group 3 received 2,000mg of the extract per kg body weight orally.

Group 4 received 1,000mg of the extract per kg body weight *per os.*

From the data collected from the above study the range of doses for LD_{50} was selected to be between 1000 to 2000mg per kg body weight of mice.

Nine mice were further treated in three groups of three each.

Group 1 received 1,000mg of the extract by kg body weight *per os*

Group 2 received 1,400mg of the extract by kg body weight *per os*

Group 3 received 1,800mg of the extract by kg body weight *per os*

From the data collected in the above studies the median Lethal dose (LD_{50}) of *Dichrostachys glomerata* extract in mice was calculated by taking the geometric mean between the lowest lethal dose that produced 100% death and the highest non-lethal dose.



Plate 3.0: Air Dried Leaves of *Psidium guajava*



Plate 3.1: Powdered Leaves of Psidium *guajava*



Plate 3.2: Air Dried Leaves of *Dichrostachys glomerata*



Plate 3.3: Powdered Leaves of *Dichrostachys glomerata*



Plate 3.4: Soxhlet Extractor

3.6.7. Post – mortem Examination of Mice Treated with Crude Ethanolic Extract of *P. guajava* and *D. glomerata*

Gross post – mortem examination was performed on all the mice in the necropsy Room, Department of Veterinary pathology and Microbiology, ABU, Zaria. Organs examined included the lungs, liver, heart, spleen, kidneys and intestine.

3.6.8. Phytochemical Tests

The freshly prepared ehtnolic extracts of *Psidium guajava* and *Dichrostachys glomerata* were subjected to standard phytochemical screening tests for various constituents (Trease and Evans, 1983; Harborne, 1998). The extracts were screened for the presence of alkaloids, tannins, glycosides, saponins, steroids, flavonoids, resins, carbohydrate and reducing sugars.

1. **Test for Alkaloids**

   Five hundred milligrams (0.5g) of the extract were stirred with 5ml of 1% aqueous HCl in a test tube. The mixture was heated for 20 minutes. It was cooled and filtered. The filtrate was used for the following tests.

(a) Two drops of Mayer's reagent were added to 1ml of the extract. Absence of a creamy precipitate in each extract tested indicated the absence of alkaloids in the extracts.

(b) Two drops of Wagner's reagent were added to 1ml of the extract. Absence of a reddish brown precipitate in each extract tested indicated the absence of alkaloids in the two extracts.

(c) To 1ml of each filtered extract was treated with 2 drops of Dragendorff's reagent. Absence of a reddish precipitate in each extract tested, indicated the absence of alkaloids in all the extract.

2. Test for Saponins

(a) Frothing Test

To 1g of the extract in a test tube was added 10ml of distilled water and shaken vigorously for 1 minute. The tube was then allowed to stand in a vertical position for 30 minutes. Honeycomb froth that persisted for 10 – 15 minutes indicated the presence of saponins.

(b) Haemolysis Test

To 1g of the extract in a test tube was added 10ml of distilled water and stirred. The solution was filtered and the filtrate was used for the test. To two separate test tubes (1 and 2) were added 2ml of 1.8% aqueous NaCl solution each. To test tube No.1, 2ml of the extract was added and to test tube No. 2 was added 2ml of distilled water. By using a syringe, 5 drops of fresh sheep blood were added to each test tube and their content gently mixed by inverting the tubes (not by shaking). The tubes were then observed for 15 – 30 minutes for haemolysis of the red blood cells. The settling down of the red blood cells (haemolysis) in test tube No. 1 confirmed the presence of saponins.

3. **Test for Tannins**

(a) Ferric Chloride Test

Five hundred milligrams (0.5g) of the extract was dissolved in 5ml of 50% alcohol; filtered through Whatmann No.1 filter paper and dispensed into two separate tubes (1 and 2). To 2ml of the filtrate in tube No. 1 was added five drops pf ferric chloride solution ($FeCl_3$). A greenish black precipitate indicated the presence of tannins.

(b) Lead Sub-acetate Test

To the next tube (tube No.2) containing the filtrate 5 drops of strong lead sub-acetate solution was added the observed coloured precipitate confirmed the presence of tannins.

4. Flavonoids

Three millilitres (3ml) of the extract was treated with 2ml of 50% ethanol. A little quantity of magnesium turnings was added to solution followed by few drops of concentrated hydrochloric acid (Conc. HCl). The red – rose colour indicated the presence of flavonoids.

5. Test for Glycosides

Five hundred milligrams (0.5g) of the extract were stirred with 10ml of boiling distilled water. This was filtered and 2ml of the filtrate hydrolyzed with a few drops of concentrated HCl and the solution rendered alkaline with a few drops of ammonia solution. Five drops of this solution were added to 2ml of Benedict's qualitative reagent and boiled. A reddish – brown precipitate showed the presence of glycosides.

6. Test for Steroids and Triterpenoids

(a) Ten millilitres (10ml) of chloroform were used to dissolve 0.5g of the extract in a test tube, 0.5mg of anhydrous sodium sulphate was added and the mixture gently mixed. The solution was filtered into two separate clean test tubes (I and II). Three millilitres (3ml) of acetic anhydride were added to test tube (No. 1) and mixed gently to remove traces of water. One millilitre (1ml) of Conc. H_2SO_4 was added down the side of the tube to form a lower layer. Blue –green colour in the upper layer and a reddish interfacial ring indicated the presence of steroids and a pink to purple colour indicated the presence of triterpenoids.

(b) Salkowski Test

One millilitre (1ml) of the extract was treated with 5 drops of concentrated H_2SO_4. The absence of red colouration in *Dichrostachys glomerata* extract indicated the absence of steroids.

7. Test for Carbohydrate

Five hundred milligrams (0.5g) of the extract were dissolved in 3ml of distilled water and heated for five minutes in a hot water bath. Five drops of Molisch's reagent were added to the solution and gently shaken. From the side of the test tube, 0.5ml of concentrated H_2SO_4 was added carefully. Reddish coloured interfacial ring indicated the presence of carbohydrate in the extract.

8. Test for Reducing Sugars

Five hundred milligrams (0.5g) of the extract were dissolved in 5ml of distilled water and filtered through a Whatmann No. 1 filter paper. The filterate was treated with 5ml of equal volume of Fehlings A and B solution and boiled in a hot water bath. Presence of a reddish precipitate indicated the presence of combined reducing sugars.

Two hundred milligrams (0.2g) of the extract were dissolved in 5ml of dilute HCl and boiled for five minutes; then 5ml of 10% NaOH was added to neutralize the acid solution. Equal volume of Fehlings A and B solution was added and boiled. The presence of brick – red precipitate confirmed the presence of reducing sugars.

3.6.9. Gastrointestinal Motility Studies in Mice

The test mice were randomly divided into eight groups (I – VIII) of five mice each. All the animals were starved for 24 hours prior to the commencement of the experiment but were allowed access to drinking, water *ad libitum.*The mice in groups (I – IV) were used to test the effect of *Dichrostachys glomerata* ethanolic extract on gastrointestinal transit, while the effect of *Psidium guajava* extract was evaluated using mice in groups (V – VIII).

Group I received 5ml of Normal saline per kg body weight intraperitoneally (i.p)

Group II received 100mg of the extract per kg body weight i.p.

Group III received 200mg of the extract per kg body weight i.p.

Group IV received 400mg of the extract per kg body weight i.p.

Five (5) minutes after drug administration, 0.2ml of a 2.5% charcoal suspension in 5% suspension of tragacanth powder was administered orally to each mouse. One hour later, all the mice were killed in gas chamber using chloroform, the abdomen opened and the entire intestine removed and immersed in formalin to halt peristalsis, the distance travelled by the charcoal plug from the pylorus to the caecum was determined with the acid of a rule and expressed as the percentage of the total length of the small intestine according to the methods of Akah (1996) and Abdullahi *et al* (2001a, b). Groups V – VIII mice were subjected to the same procedures. Group V received 5ml of normal saline per kg body weight i.p. while groups VI, VII and VIII received graded doses of *Psidium guajava* extract (100, 200, and 400mg/kg i.p.), respectively. Determination of the gastrointestinal transit was as in groups I – IV.

3.7.0. Studies on Castor Oil - Induced Diarrhoea in Rats

The rats were fasted for 16 hours prior to the commencement of the experiment and randomly divided into 10 groups (I – X) of five rats each. The rats in groups (I to V) were used to evaluate the effect of *Dichrostachys glomerata* extract on castor –oil induced diarrhoea while groups (VI to X) were used for the evaluation of the effect of *Psidium guajava* extract.

Group I received 5ml of normal saline per kg body weight orally (p.o).

Group II received 100mg of the extract per kg body weight (p.o).

Group III received 200mg of the extract per kg body weight orally (p.o).

Group IV received 400mg of the extract per kg body weight orally (p.o).

Group V received 5mg of loperamide per kg body weight (p.o).

After 30 minutes of drug pre – treatment the rats were challenged with castor – oil, 0.5ml/rat orally and placed in individual cage over clean white paper.

The cages were examined at 15 minutes intervals for a 4 hours period and the feaceal outputs recorded at each interval and scored as follows:

1 = Normal pelleted faeces

2 = soft formed faeces

3 = watery feaces and/or diarrhoea as prescribed by Vogel and Vogel (1997).

After 4 hours of Castor – oil challenge, the rat cages were inspected (by conserves unaware of the particular treatment) for the presence of the characteristic diarrhoea droppings. The absence of diarrahoea faeces was recorded as a protection from diarrhoea and the percentage protection calculated (Akah, 1996; Abdullahi *et al.*, 2001a, b).

Psidium guajava extract was evaluated for antidiarrhoeal effect using the same procedure as described above.

3.7.1. Antibacterial Screening of *P. guajava* and *D. glomerata*

a. The method used in this study was the punch Hole Method (Cruckshank *et al.*, 1975; Ejimadu and Ogbeide, 2001) in which a uniform and equidistant hole of 5mm in diameter was punched into the inoculated nutrient agar using a sterile cork borer.

b. Preparation of the Medium

Nutrient agar medium was prepared by dissolving 2.8g of nutrient agar in 100ml of distilled water. The solution was sterilized in an autoclave at 121^0C at 1.1 normal pressures for 15minutes. It was cooled and poured into sterile Petri dishes to gel. The agar depth of the medium was 4mm.

c. Preparation of the cultures and inoculation

Pure cultures of *Staphylococcus aureus, Salmonella typhi and Escherichia coli* obtained from the Bacteriology Department, National Veterinary Research Institute, Vom, Nigeria were separately used to inoculate the Petri-dishes. This was done by streaking the surfaces of the plates in a zigzag manner until all the surface of the plates was covered. The inoculated plates were incubated at room temperature for 24 hours (Ejimadu and Ogbeide, 2001).

d. Assay of Extracts

Uniform and equidistant holes of 5mm in diameter were punched into the inoculated nutrient agar using a sterile cork borer. The bottoms of the holes were sealed with a drop of molten agar before introducing the plant extracts (200mg, 100mg and 50mg/ml) and the reference drug (Ampicillin) to prevent percolation (leaking).

The plates were left on the bench for 30 minutes to allow diffusion of the extract into the medium and then incubated at 37^0C for 24 hours. The zones of inhibition were measured by the use of transparent plastic rule and recorded.

3.7.2. Evaluation of anti-Inflammatory Property of *Psidium guajava* and *Dichrostachys glomerata* Extract in Rats

This was done by using fresh chicken egg albumen – induced rat hind paw oedema (Ekpendu *et al.,* 1998a, b; Muko and Ohiri, 2000; Oyewole, 2004) as a model of acute inflammation. Young adult Wistar rats (12 – 15 weeks old) weighing between 180 – 200g were used. The animals were divided into eight groups (1 – 8) of eight rats per group.

Groups 1 – 5 were used to access the anti-inflammatory effect of *Psidium guajava* extract while Groups 6 – 8 were the models for *Dichrostachys glomerata* extract evaluation.

Group 1 received 5ml per kg body weight of distilled water orally (p.o.)

Group 2 received 200mg of the extract per kg body weight p.o.

Group 3 received 400mg of the extract per kg body weight p.o.

Group 4 received 800mg of the extract per kg body weight p.o.

Group 5 received 10mg of indomethacin orally.

The same doses were maintained for the evaluation of *Dichrostachys glomerata* (Groups 6 – 8). Rat hind paw oedema was induced by intra-planter injection of fresh chicken egg albumen (0.5ml per kg body weight), a cheap phogistic agent (Ekpendu *et al.,* 1994; Muko and Ohiri, 2000; Oyewole, 2004). The right hind paw acute inflammation was induced in each rat by injecting 0.5ml/kg of fresh chicken egg albumen into the sub-plantar surfaces.

Inflammation was evident 5 – 10 minutes following the sub-plantar injection. Measurement of paw size was carried out by wrapping a piece of cotton thread round the paw and measuring the circumference with a meter rule. The measurement of the paw size was carried out for 3 hours at 30 minutes interval (30, 60, 90, 120, 150, 180 minutes following fresh egg albumen administration. The inhibitory activity was calculated using the formula $Co\text{-}C_t/C_o \times 100$ (Oyewole, 2004).

C_o = is the average inflammation (hind paw oedema of control (Group 1) rats at a given time and

C_t = is the average inflammation of (Groups 2, 3, 4, 6, 7, 8) and 5 rats treated with plant extracts respectively, at the same time.

3.8.0. Isolated Tissue Studies

3.8.1. Preparation of solution for Isolated Tissue Studies

A highly concentrated solution of the extract was prepared in deionized water. The reference drugs were prepared by weighing and dissolving in the required volumes of deionized water to obtain the desired concentration of the drugs. Serial dilutions of the stock solutions were made in order to get the desired concentration. Dilutions from the stock were made fresh for each experiment. Both the extract and reference drugs were stored at 4^0C until use. Tyrode solution was prepared fresh for every days experiment.

3.8.2. Preparation of Rabbit Jejunum and Guinea Pig Ileum

The modified Magnus technique as reported by Vogel and Vogel (1997) was used. Six rabbits (700 – 1500g) and five guinea pigs (200 – 315g) of either sex were starved of food overnight (16 hours), stunned and a midline incision made at the abdomen to expose the small intestine. The intestines were gradually removed and the sections of the rabbit jejunum and guinea pig ileum were cut, respectively. Suitable lengths (2 - 3cm) were fixed with a tissue clamp and suspended in 25ml organ bath containing Tyrode's solution. The solution was oxygenated with air bubbles using an air pump and maintained at 37^0C using thermocirculator. The lower end of the tissue was attached to an oxygenated tube, while the upper end was fixed to an isometric force transducer. A preload of 1g was chosen. The transducer was connected to a microdynanometer, set at a paper speed of 24mm per minute and sensitivity of 2, for recording of responses. After a pre-incubation time of 60 minutes, the experiments were started.

3.8.3. Application of Test Substances to the Isolated Rabbit Jejunum and Guinea Pig Ileum

Calculated volumes of the constituted extract and the drug solutions were measured and introduced into the organ bath containing the tissues, using 1ml (insulin) syringes. The

test substances were allowed to be in contact with the isolated tissues for 30 seconds, after which the tissues were washed three times by emptying and refilling the organ bath with fresh Tyrode's solution. The paper speed button on the recording microdynanometer was switched off before washing of tissues, and the tissues were allowed to recover before it was switched on. The tissue was allowed a resting period of 10 minutes before the next addition.

3.8.4. Effects of the Crude Ethanolic Extracts of *P. guajava* and *D. glomerata* Leaves, Agonists and Antagonists on the Isolated Rabbit Jejunum and Guinea Pig Ileum

Dose – response effects were obtained for acetylcholine and extract solution on rabbit jejunum. The effect of atropine (an antagonist of ACh at muscarinic receptor sites) on the response of the isolated rabbit jejunum was investigated. The effects of the interaction between ACh and the extracts on the isolated smooth muscles were also investigated.

On the isolated guinea pig ileum, dose response effects were obtained for histamine and the extract solution. The effect of the interaction between histamine and the extracts were equally investigated as well as the effect of mepyramine (a histamine antagonist) on the isolated smooth muscles.

Responses were recorded isometrically on an Ugo Basile unrecorded 7050 (Amos *et al.*, 1998; Abdullahi *et al.*, 2001a, b; Dzenda, 2004). Solutions were used on the isolated smooth muscle tissues of rabbit jejunum and guinea pig ileum. The concentration of ACh used to investigate the effect of the extracts on the isolated smooth muscles was 8.0 – 2.0 $x10^{-2}$ µg/ml; histamine, 1.0 x 10^{-1} - 4 x 10^{-1} mg/ml; atropine, 1.3 x 10^{-1}mg/ml and mepyramine, 7.2 x 10^{-4} µg/ml.

3.8.5. Evaluation of Anthelmintic Properties of *P. guajava* and *D. glomerata*

a. Faecal Egg Count (FEC) (Modified McMaster Technique)

- Three grams (3g) of faeces were weighed from a well mixed sample into a 4oz jar.
- Forty-two millilitres (42ml) of saturated salt (NaCl) solution were measured out using a 50ml measuring cylinder and added to the faeces in the 4oz jar.
- With the aid of a thick glass rod the faeces was broken and emulsified in the salt solution ensuring an even suspension.
- The suspension was stirred thoroughly and quickly poured through a clean coffee strainer into a clean 4oz jar.
- The faecal residue on the strainer was discarded and the filtrate brought to 45ml volume by adding more salt solution.
- The jar was closed with tight fitting lid, pressed down firmly and the jar inverted three times to mix its contents without shaking.
- With a wide – bore Pasteur pipette, the two chambers of the McMaster slide were filled one after the other and placed on the microscope stage for two minutes to allow the eggs rise in the salt solution and rest against ruled surface of the McMaster slide.
- The eggs present within the ruled square of both chambers were focused and counted using low power objective (X10) of the microscope.
- The egg per gram (EPG) was calculated as follows:

  EPG = Total number of eggs in both chambers (N) x 50

b. Treatment and Follow-up Procedures

Prior to treatment, faecal samples were obtained per rectum from each animal. On day 0, the goats were blocked by sex and allocated, within sex to eight treatment groups according to complete randomized design taking into cosideration their live weights. The goats were penned by treatment group until the end of the study. On day 1, the experimental groups II, III and IV were drenched with graded doses of the ethanolic crude extract of *Psidium guajava* 200, 400 and 800 mg/kg/body weight, respectively, while group 1 served as untreated control. The treatment was repeated the second day. Groups VI, VII and VII goats were drenched with graded doses of *Dichrostachys glomerata* crude extract 200, 400 and 800 mg/kg body weights, respectively, while group V was treated with albendazole (25mg/kg). The treatment was not repeated the second day. Observation of clinical signs and death was undertaken twice daily. Faecal egg counts were performed on each animal on days 0, 6, 12, 18, 24, 30, and 36 post-treatment.

3.8.6. Evaluation of Results

The acute toxicity (the median lethal dose (LD_{50}) of the extract were estimated in Swiss albino mice orally by modified Locke's method. Results are expressed as means ± standard error of the means (Means ± SEM) where `n` is the number of observations in the group. The data on castor oil-induced diarrhoea were analyzed by Chi – square test and where applicable, comparison between groups were made using one-way analysis of variance (ANOVA) (Snedecor and Cochran, 1980). Values of $P<0.05$ were considered significant.

CHAPTER FOUR

4.0 RESULTS

4.1.1 Preparation of Ethanolic Extract of *P. guajava* Leaves

The initial weight of the fresh *P. guajava* leaves was 1.215kg, while the final dry weight was 0.405kg (405g), representing 66.79% moisture content and 33.3% dry matter w/w. The total amount of ground *P. guajava* leaves used for extraction was 400g. Upon evaporation of the filtrate after extraction a greenish brown substance was obtained; 400g of the sample yielded 70.5g, constituting about 17.6% of the original sample by weight. Percentage yield was calculated as follows:

$$\% \text{ Yield} = \frac{\text{Weight of extract (concentrated)}}{\text{Weight of powdered leaves taken}} \times 100\%$$

$$\% \text{ Yield} = \frac{70.5\text{g} \times 100}{400\text{g}}$$

$$= 17.6\%.$$

4.1.2. Preparation of Ethanolic Leaf Extract of *D. glomerata*

The initial weight of the fresh *Dichrostachys glomerata* leaves was 1.126kg, while the final dry weight was 0.416kg (416g), representing 63.03% moisture content and 36.97% dry matter w/w. The total amount of ground *Dichrostachys glomerata* leaves used for extraction was 400g. Upon evaporation of the filtrate after extraction a brownish substance was obtained, 400g of the sample yielded 72.0g, constituting about 18.0% of the original sample by weight. Percentage yield was calculated as follows:

$$\% \text{ Yield} = \frac{\text{Weight of extract (concentrated)}}{\text{Weight of powdered leaves taken}} \times 100\%$$

$$\% \text{ Yield} = \frac{72.0\text{g} \times 100}{400\text{g}}$$

$$= 18.0\%$$

4.1.3 Acute Oral Toxicity studies of Crude Ethanolic Leaf Extract of *P. guajava* in Mice

The extract was completely soluble in distilled water, after rigorous stirring with a glass rod. Therefore, distilled water was used as solvent for acute toxicity studies.

The results of the pilot tests were summarized as shown in Table 4.0. From the result, 10, 000mg/kg caused 100% mortality ($^3/_3$) while 1000 mg/kg, 100 mg/kg and 10 mg/kg caused 0% mortality ($^0/_3$). Results from further studies (Tables 4.1 and 4.2), showed that concentrations of 4000, 3000, 2000 and 1900mg/kg all caused 100% mortality (3/3), whereas 1,600mg/kg caused 0% mortality ($^0/_3$). Clinical signs of toxicity included initial excitation, restlessness, gasping and jerking, followed by depression, recumbency and coma. Signs appeared 50 seconds after extract administration. Within 30 – 60 minutes most of the mice treated with 2,000 – 10,000mg/kg of the extract were in coma. Death occurred within 60minutes to 24hours. Clinical signs in the mice administered with 1,900mg/kg were milder but they died within 24h period. The mice that received only the vehicle - distilled water showed no clinical signs. Taking the geometric mean from the sum of the least lethal dose (1,900mg/kg) and the nearest non - lethal dose to it (1,600mg/kg), the LD_{50} was calculated to be 1750 ± 212mg/kg.

4.1.4 Acute Oral Toxicity Studies of Crude Ethanolic Leaf Extract of *D. glomerata* in Mice

The extract was completely soluble in distilled water, after rigorous stirring with a glass rod; and so distilled water was used as solvent for dissolving the extract used in acute toxicity studies. The results of the pilot tests were summarized (Table 4.3). The extract at the 10,000mg/kg dose caused 100% mortality (3/3) while 1,000mg/kg, 100mg/kg and 10mg/kg doses caused 0% mortality (0/3). Results from further studies showed that at the doses of 4,000, 3,000, 2,000 and 1,800mg/kg body weight, the extract caused 100% mortality (3/3) whereas 1,400mg/kg caused 0% mortality (0/3) (Tables 4.4 and 4.5).

Clinical signs of toxicity included initial excitation, staggering, restlessness, burrowing, protrusion of the eyes, depression and recumbency. The signs appeared at 70 seconds of extract administration and within 30 – 90 minutes, most of the animals treated with 1,800 – 10,000mg/kg were in coma. Death occurred within 60minutes – 24hours. Clinical signs of toxicity in the mice that received 1,800mg/kg of the extract were relatively mild. The mice that received only the vehicle – distilled water showed no signs of toxicity. Taking the geometric mean from the sum of the least lethal dose (1,800mg/kg) and the nearest non-lethal dose to it, 1400 mg/kg, the LD_{50} was calculated to be 1600 $\pm$ 283 mg/kg.

Table 4.0. Results of the Pilot Test for LD_{50} of the Ethanolic Leaf Extract of *Psidium guajava* in Mice

Group	Dose (mg/kg)	No. Dead/No. Treated	Clinical Signs
1	10,000	$^{3}/_{3}$	Excitation, restlessness, Jerking, burrowing, depression gasping, recumbency, death
2	1,000	$^{0}/_{3}$	-
3	100	$^{0}/_{3}$	-
4	10	$^{0}/_{3}$	-
5	Control	$^{0}/_{3}$	-

Table 4.1 Test for LD_{50} of Ethanolic Extract of *Psidium guajava* Leaf

Group	Dose (mg/kg)	No. Dead/No. Treated	Clinical Signs
1	4,000	$^{3}/_{3}$	Excitation, restlessness, gasping, burrowing, depression, coma death.
2	3,000	$^{3}/_{3}$	-
K3	2,000	$^{3}/_{3}$	-
4	1,000	$^{0}/_{3}$	-

Table 4.2 Determination of LD_{50} of Ethanolic Leaf Extract – of *Psidium guajava* Leaf

Group	Dose (mg/kg)	No. Dead/No. Treated	Clinical Signs
1	1,000	$^{0}/_{3}$	-
2	1,300	$^{0}/_{3}$	-
3	1,600	$^{0}/_{3}$	-
4	1,900	$^{3}/_{3}$	Excitation, gasping, restlessn burrowing, recumbency, dea

Table 4.3. Results of the Pilot Test for LD_{50} of Ethanolic Leaf Extract of *Dichrostachys glomerata*

Group	Dose (mg/kg)	No. Dead/No. Treated	Clinical Signs
1	10,000	$^{3}/_{3}$	Restlessness, excitation, gasping, protrusion of the eyes, depression, recumbency, death.
2	1,000	$^{0}/_{3}$	-
3	100	$^{0}/_{3}$	-
4	10	$^{0}/_{3}$	-
5	Control	$^{0}/_{3}$	-

Table 4.4: Test for LD_{50} of Ethanolic Leaf Extract of *Dichrostachys glomerata*

Group	Dose (mg/kg)	No. Dead/No. Treated	Clinical Signs
1	4,000	$^{3}/_{3}$	Restlessness Excitation, dilation of the pupils, gasping depression, recumbency, death
2	3,000	$^{3}/_{3}$	"
3	2,000	$^{3}/_{3}$	"
4	1,000	$^{1}/_{3}$	-

Table 4.5: Determination of LD_{50} of Ethanolic Leaf Extract of *Dichrostachys glomerata*

Group	Dose (mg/kg)	No. Dead/No. Treated	Clinical Signs
1	1,000	$^{0}/_{3}$	-
2	1,400	$^{0}/_{3}$	-
3	1,800	$^{3}/_{3}$	Excitation, dilation of the pupils, depression, burrowing recumbency.

4.1.5 Gross Post-Mortem Findings

Gross post mortem examination of the mice that died as a result of the acute toxicity of the extracts of *P. guajava* and *D. glomerata* showed congestion of the lungs, liver, kidneys and spleen, enteritis was prominent in *Dichrostachys glomerata* toxicity. Hepatic hemorrhage was observed in the mice that died of *P. guajava* administration. No gross lesions were observed in control animals and those that survived lower doses of extracts administration.

4.1.6 Phytochemical Analysis

Preliminary phytochemical screening results showed that steroids and alkaloids were absent in the ethanolic extracts of *Dichrostachys glomerata* and *Psidium guajava* leaves. Tannins, flavonoids, saponins, resins, carbohydrate, reducing sugars and triterpenoids tests were positive for both plant extracts, while only the extract of *Psidium guajava* tested positive for glycosides. Tannins and flavonoids were highly positive in the extracts of *Psidium guajava* and *Dichrostachys glomerata* (Table 4.6).

Table 4.6: Phytochemicals in the Crude Ethanolic Leaf Extracts of *Psidium guajava* and *Dichrostachys glomerata*

Phytochemicals (Test)	Plant Extracts	
	Dichrostachys glomerata	***Psidium guajava***
Alkaloids	-ve	-ve
Tannins	+++	+++
Glycosides	-ve	+
Saponins	++	++
Steroids	-ve	-ve
Flavonoids	+++	+++
Resins	++	++
Triterpenoids	+	+
Carbohydrate	++	++
Reducing sugar	+	+

Key:

-ve = Negative

\+ = Mildly present

++ = Moderately present

+++ = Highly present

4.1.7. Effect of Varying Concentrations of the Ethanolic Leaf Extracts of *P. guajava* and *D. glomerata* on Contractile Activity of Isolated Rabbit Jejunum (n = 3)

Before the application of the test substances and acetylcholine the isolated rabbit jejunum produced spontaneous pendular contraction (frequency of 12.33 $\pm$ 0.57 per minute and amplitude of 25.43$\pm$ 0.10 (n = 3) (Fig. 4.0). The isolated smooth muscle of rabbit jejunum was sensitive to ACh, maximum response was obtained at 8.0 x 10^{-2}mg/ml. The responses of the isolated smooth muscle preparation to ACh were dose dependent (Fig 4.1).

The isolated smooth muscle preparation of rabbit jejunum relaxed dose dependently on the application of ethanolic crude leaf extracts 0.4 mg/ml -3.2 mg/ml of *P. guajava* and *D. glomerata* (Figs 4.2 and 4.3). The maximum reduction in both the amplitude and frequency of contraction occurred with 3.2 mg/ml of both extracts (Figs 4.2 and 4.3). At this concentration of the extract in the medium, the amplitude and frequency of contraction reduced maximally. The crude ethanolic leaf extract of *P. guajava* and *D. glomerata* (3.2 mg/ml) like atropine (1.3 x 10µg/ml) abolished the spontaneous rhythmic contraction of the isolated rabbit jejunum (Figs 4.2, 4.3 and 4.4).

The extracts of *P. guajava* and *D. glomerata* (0.4mg – 3.2mg) also dose dependently relaxed Ach (8.0 x 10^{-2}µg/ml) induced contractions of isolated rabbit jejunum (Fig. 14.0). The extracts of *P. guajava* and *D. glomerata* (3.2 mg/ml) produced maximal attenuation of 89% and 81% respectively of the ACh (8.0 x 10^{-2}µg/ml) induced rhythmic contraction of isolated rabbit jejunum (Tables 4.7 and 4.8; Fig. 4.5).

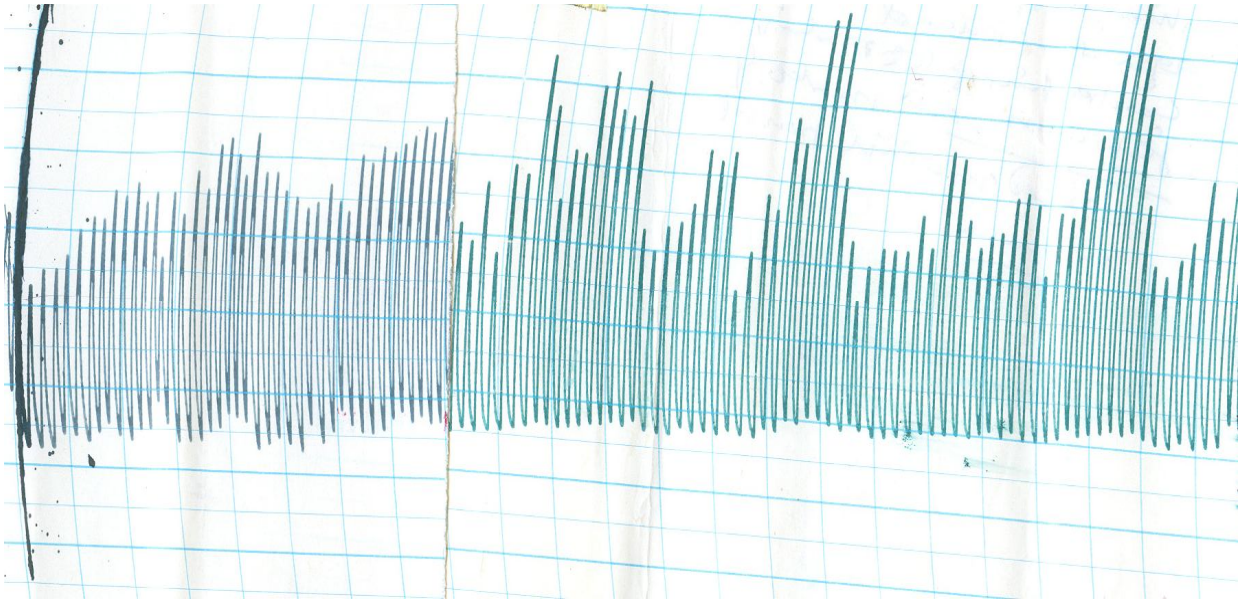


Fig. 4.0: Normal pendular spontaneous contraction of isolated rabbit jejunum

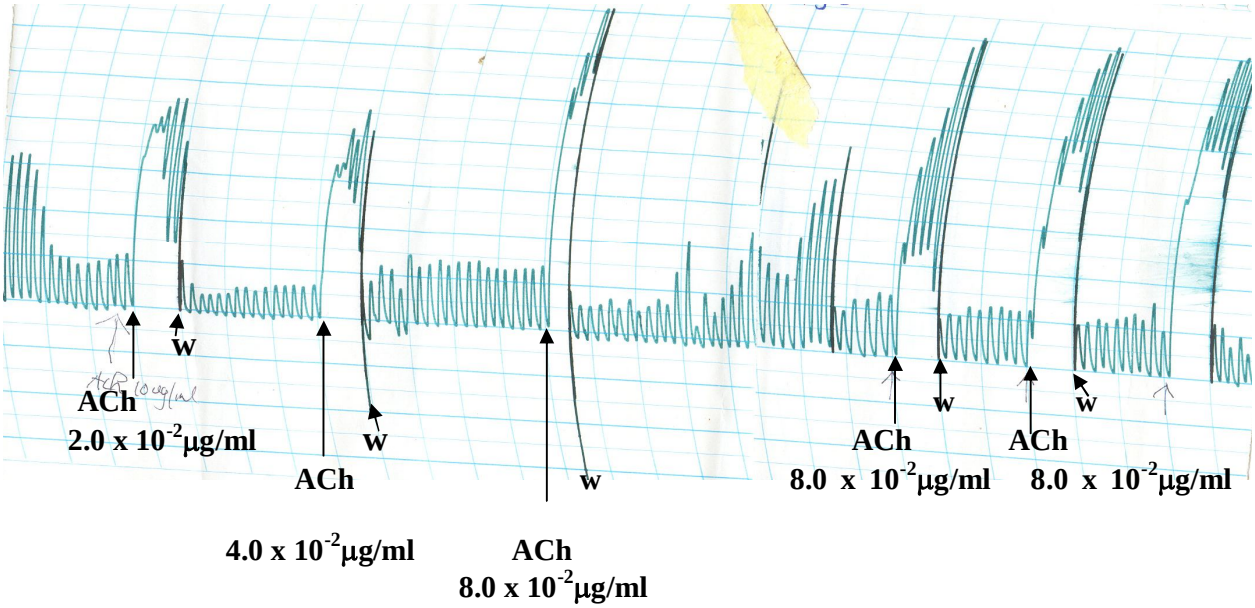


Fig 4.1: **Effects of acetylcholine on spontaneous contraction of isolated rabbit jejunum**

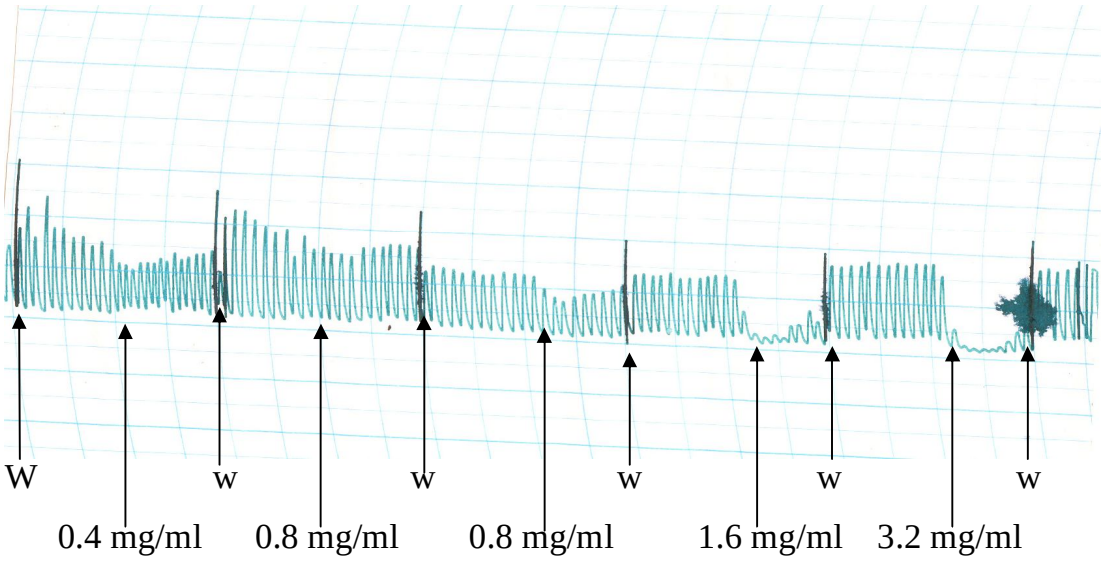


Fig 4.2: **Concentration-dependent effects of *Psidium guajava* ethanolic leaf extract on the frequency and amplitude of contraction of isolated rabbit jejunum**

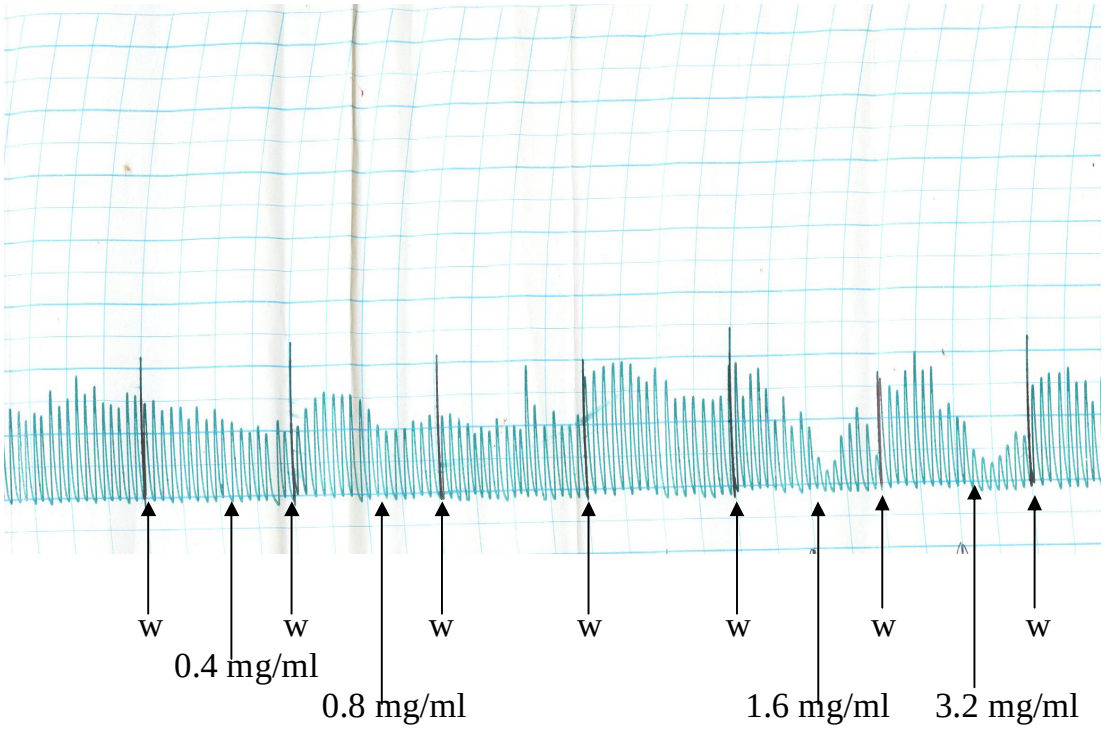


Fig 4.3: Concentration-dependent effects of *Dichrostachys glomerata* ethanolic leaf extract on the frequency and amplitude of contraction of isolated rabbit jejunum

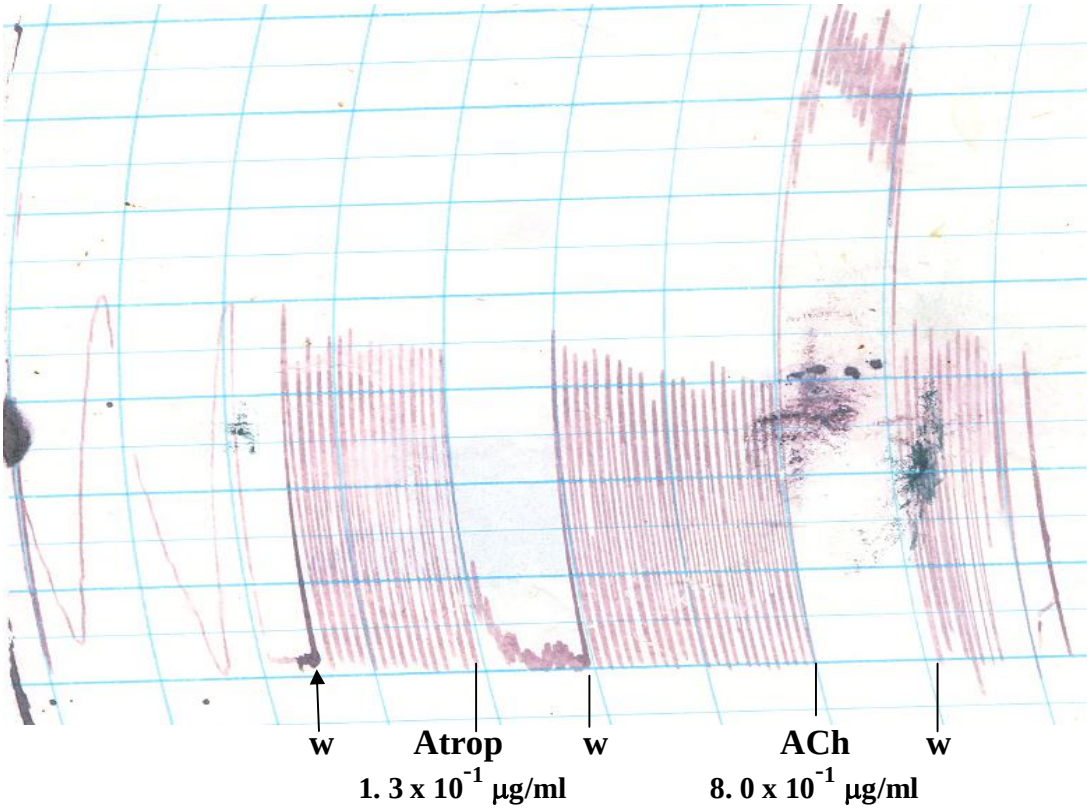


Fig 4.4: Effects of atropine and acetylcholine (ACh) on the spontaneous pendular contraction of isolated rabbit jejunum

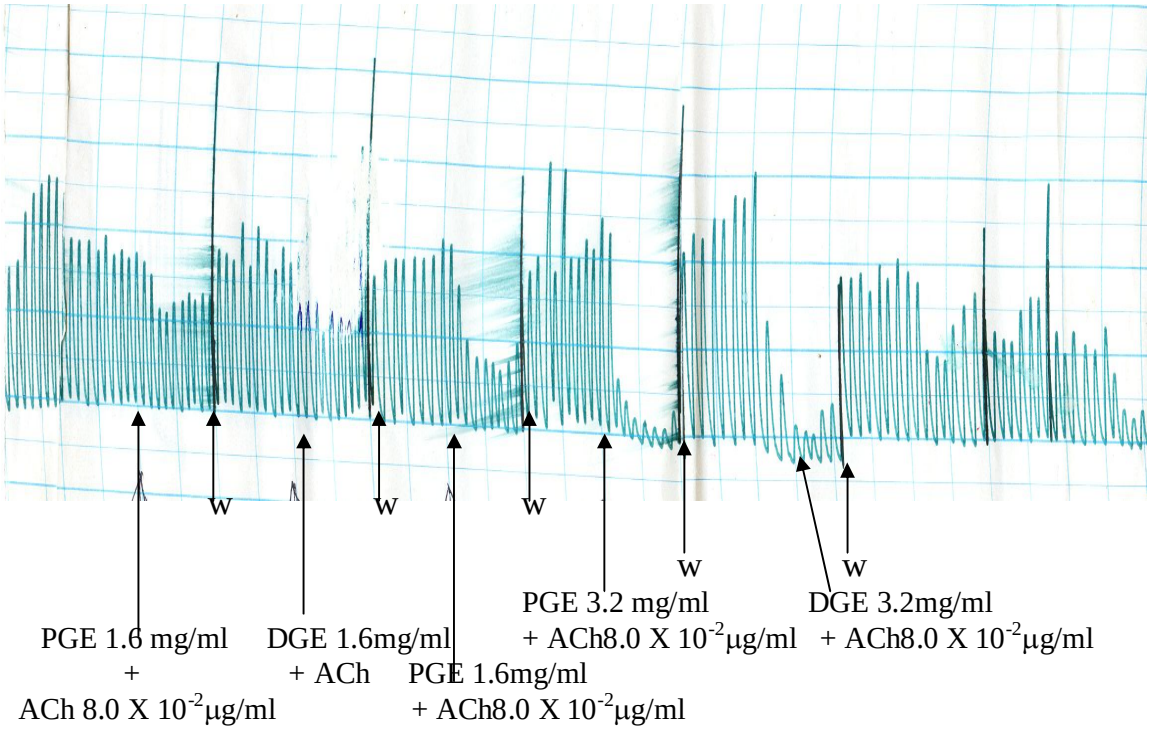


Fig 4.5: Inhibitory effects of *P. guajava* and *D. glomerata* on ACh induced contraction of isolated rabbit jejunum

Table 4.7: The Inhibitory Effect of the Ethanolic Leaf Extract of *D. glomerata* (DGE: 0.4 – 3.2 mg/ml) and Atropine (1.3×10^{-1} µg/ml) on Acetylcholine (8.0×10^{-2} µg/ml) Induced-Contractions of Rabbit Jejunum (n = 3; mean ± SEM)

Test substances	Concentrations	Amplitude of Contraction (mm)	Percentage of Blockade of ACh Atropine and the Extracts (%)
ACh (8.0×10^{-2}ug/ml)		34.59 ±0.21	
Normal contraction (control)		25.43 ± 0.1	
Atropine (1.3×10^{-1})	+ACh (8.0×10^{-2})	1.90 ± 0.2	94.6*
DGE (0.4 mg/ml)	+ ACh (8.0×10^{-2})	23.12 ± 1.2	34.7*
DGE (0.8 mg/ml)	+ ACh (8.0×10^{-2})	18.33 ± 0.5	48.2*
DGE (1.6 mg/ml)	+ ACh ($8.0\ 10^{-2}$)	15.07 ± 0.3	57.4*
DGE (3.2 mg/ml)	+ ACh (8.0×10^{-2})	6.70 ± 0.6*	81.1*

n = Number of Animals used.

SEM = Standard error of mean;

* = $P < 0.05$ = significant difference

ACh = Acetylcholine

DGE = *Dichrostachys glomerata* Extract

PGE = *Pisdium guajava* Extract

Table 4.8: Inhibitory Effect of the Ethanolic Leaf Extract of *P. guajava* (PGE: 0.4 – 3.2 mg/ml) and Atropine (1.3 x 10^{-1}μg/ml) on Acetylcholine (8.0 x 10^{-2}μg/ml) Contractions of the Rabbit Jejunum (n = 3 mean ± SEM

Test Substances	Concentrations	Amplitude of Contraction (mm)	Percentage Blockade of ACh Atropine and the Extracts (%)
ACh (8.0 x 10^{2}ug/ml)		34.59 ±0.21	
Normal contraction (control)		25.43 ± 0.1	
Atropine (1.3 x 10^{-1}) μg/ml	+ ACh (8.0 x 10^{-2})	1.90 ± 0.2	94.5*
PGE (0.4) mg/ml	+ ACh (8.0 x 10^{-2})	23.10 ± 0.1	33.9*
PGE (0.8) mg/ml	+ ACh (8.0 x 10^{-2})	15.33 ± 0.5	55.8*
PGE (1.6) mg/ml	+ Ach (8.0 x 10^{-2})	11.90 ± 0.2	56.7*
PGE (3.2) mg/ml	+ Ach (8.0 x 10^{-2})	3.83 ± 0.3*	89.0*

n = Number of animals:

SEM = Standard error of mean;

* = $P<0.05$ = significant difference

ACh = Acetylcholine

PGE = *Pisdium guajava* Extract

Table 4.9: Doses of Agonists Producing Maximal Dose Related Contraction Responses on isolated intestinal smooth muscles and concentrations of corresponding antagonists reverting those Actions

Agonists	Dose (μg/ml)	Antagonist	Dose (μg/ml)	Number of Experiments (n)
Acetylcholine	8.0×10^{-2}	Atropine	(1.3×10^{-1})	3
		P. guajava Extract	(32.0×10^{2})	3
		D. glomerata Extract	(32.0×10^{2})	3
Histamine	4.0×10^{-1}	Mepyramine	(7.2×10^{-4})	3
		P. guajava Extract	(32.0×10^{2})	3
		D. glomerata Extract	(32.0×10^{2})	3

4.1.8 Contractile Response of the Guinea Pig Ileum to Varying Concentrations of Histamine and the Ethanolic Extracts of *P. guajava* and *D. glomerata*

The isolated Guinea pig ileum produced no apparent spontaneous contractile activity (Fig. 4.6). Recordings on the microdynanometer produced a straight line. No rhythmic contractions were observed in the guinea pig ileum before addition of the histamine (Fig 4.7).

Histamine ($1.0 \times 10^{-4} - 4.0 \times 10^{-4}$ µg/ml) induced the contraction of the guinea pig ileum, the contractions were concentration dependent, and maximum contraction occurred at the concentration of 4.0×10^{-4} µg/ml (Fig 4.8). After each washout (W) of histamine in the medium, histamine- induced contractions of the isolated guinea pig ileum were completely abolished (Figs 4.7 and 4.8).

Application of *P. guajava* and *D. glomerata* leaf extracts (0.4 – 3.2mg/ml), respectively, to the bathing medium did not elicit any contractile effect on the guinea pig ileum. Histamine (4.0×10^{-4} mg/ml) induced contractions of the isolated guinea pig ileum were attenuated by the extracts of *P. guajava* and *D. glomerata* in a dose – related manner (Fig. 4.9). Histamine (4.0×10^{-4}µg/ml) induced contraction of the guinea pig ileum was completely abolished by mepyramine (7.2×10^{-4}µg/ml) (Fig. 4.10). The extracts of *P. guajava* and *D. glomerata* like mepyramine induced spasmolytic (tissue relaxing) effects on the isolated guinea pig ileum dose dependently (Figs 4.9 and 4.10; Table 4.9).

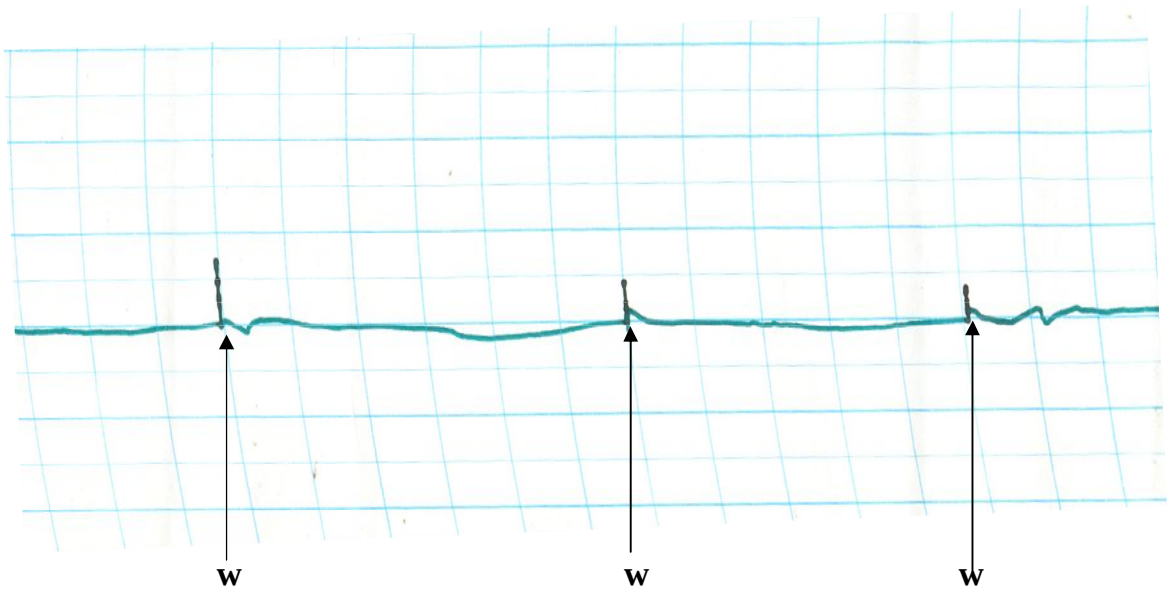


Fig 4.6: Normal contraction of isolated guinea pig ileum

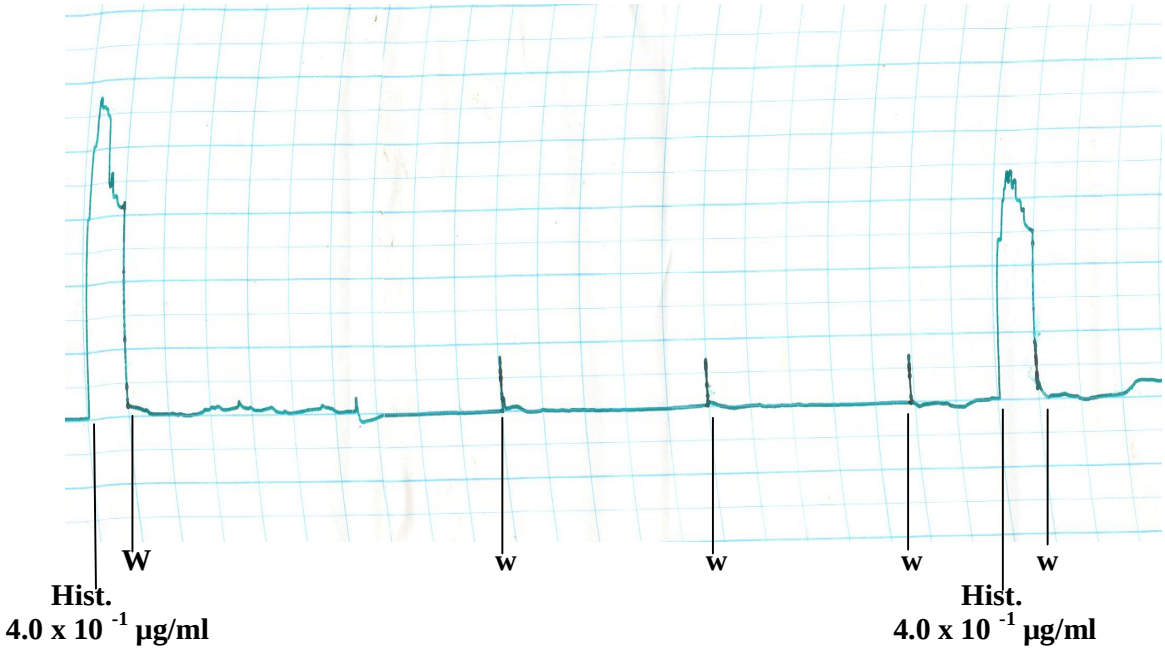


Fig 4.7: Histamine – induced contraction of isolated guinea pig ileum

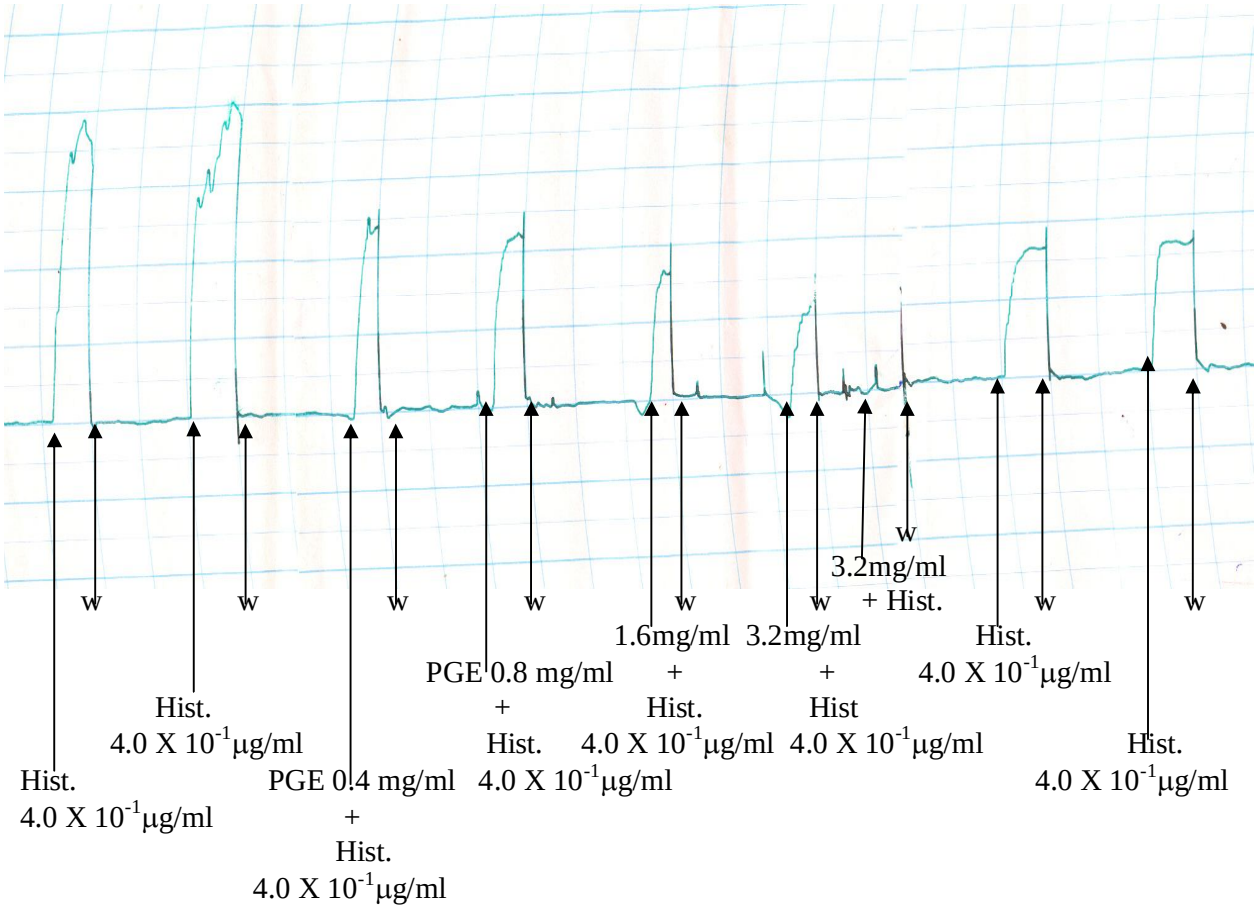


Fig 4.8: **Effects of *Psidium guajava* leaf extract on histamine – induced contraction of isolated guinea pig ileum**

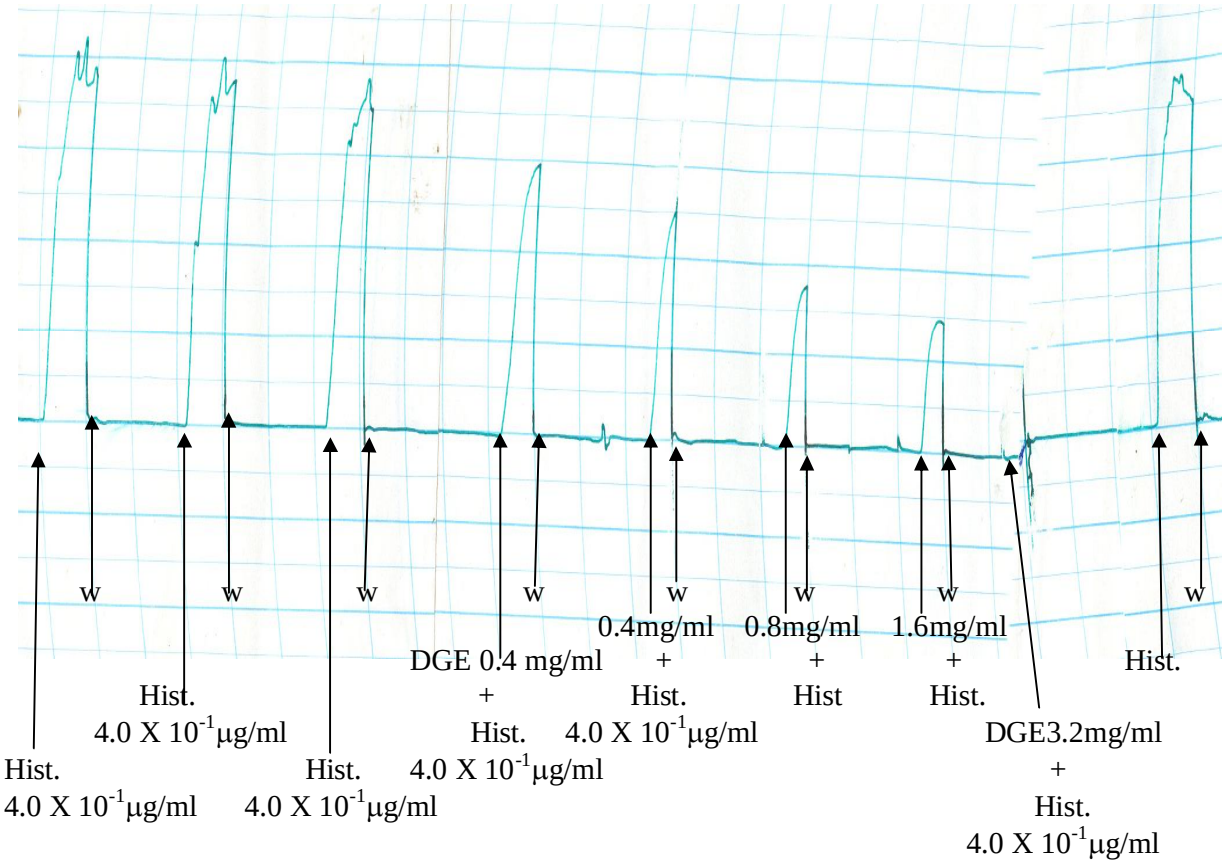


Fig 4.9: Effects of *Dichrostachys glomerata* ethanolic leaf extract on histamine –induced contraction of isolated guinea pig ileum

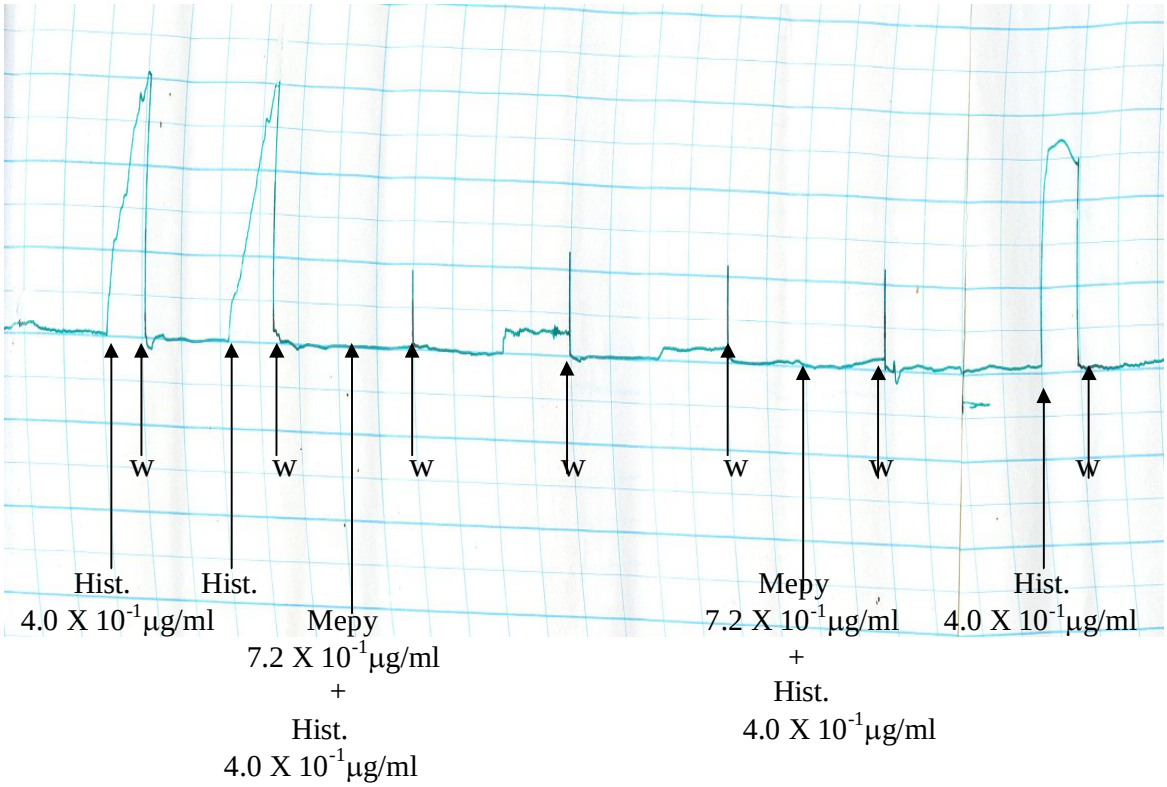


Fig 4.10: **Inhibitory effects of mepyramine on histamine induced contraction of isolated guinea pig ileum**

4.1.9 Anti-inflammatory Screening

Sub plantar injections of fresh egg albumen (0.5ml/kg) produced marked, sustained, time related and progressive increases in the hind paw diameters of the control, untreated rats. Maximal swelling and/or oedema occurred 90 minutes following fresh egg albumen administration. The ethanolic leaves extracts of *P. guajava* and *D. glomerata* (200 – 800mg/kg per os) produced dose and time – related, sustained and significant ($P<0.005$) reduction in the fresh egg albumen – induced acute inflammation of the rats, hind paw oedema, just like the reference drug (indomethacin 10mg/kg) (Tables 4.10 and 4.11). Very significant results were observed at the highest dose of the extracts (800mg/kg) at the third hour after fresh egg albumen injection with (95 and 98%) reduction of the paw oedema, respectively. Distilled water alone did not modify the fresh egg albumen-induced rat paw oedema.

Table 4.10: Effect of the Ethanolic Leaf Extract of *P. guajava* on fresh egg Albumen – Induced Paw Oedema in Rats (n = 8, Mean ± SEM)

		Time in minutes and Paw Oedema in mm					
Treatment	**Dose (p.o.)**	30 Minutes	60 Minutes	90 Minutes	120 Minutes	150Minutes	180 Minutes
Distilled water (control)	5ml/kg	9.86±0.25 (mm)	11.08±0.47 (mm)	13.03±0.24 (mm)	13.80±0.16 (mm)	14.39±0.15 (mm)	1.478±0.14 (mm)
PGE	200mg/kg	6.28±0.23 (36.6%)	5.14±0.18* (56.55)	4.10±0.16** (68.5%)	3.49±0.28** (73.2%)	2.57±009** (84.4%)	1.49±0.08*** (89.9%)
PGE	400mg/kg	4.92±0.16* (50.1%)	4.31±0.24* (61.1%)	3.70±0.19** (71.65)	2.85±0.14** (79.4%)	1.70±017*** (88.2%)	0.94±0.15*** (93.6%)
PGE	800mg/kg	4.46±0.07* (54.8%)	3.99±0.13* (64.0%)	3.48±007** (73.5%)	2.72±0.19** (80.0%)	1.28±0.12*** (91.0%)	0.16±004*** (98.9%)
Indomethacin	10mg/kg	4.35±0.10* (55.6%)	3.91±0.13* (64.7%)	3.19v0.14** (75.5%)	2.42±0.05** (82.5%)	0.96±0.05*** (93.4%)	0.05±0.02*** (99.7%)

n = Number of animals; SEM = standard error of the mean;

* = $P<0.05$ - Significant difference compared with control values;

** = $P<0.01$ - Very significant difference; and

*** = $P<0.001$-Very highly significant difference. Each quoted value represents the mean (± SEM) of eight observations. Inhibition of the egg albumen-induced inflammation by the plants extract and the reference drug (indomethacin) used are indicated as percentage (%)

PGE = *Psidium guajava* Extract

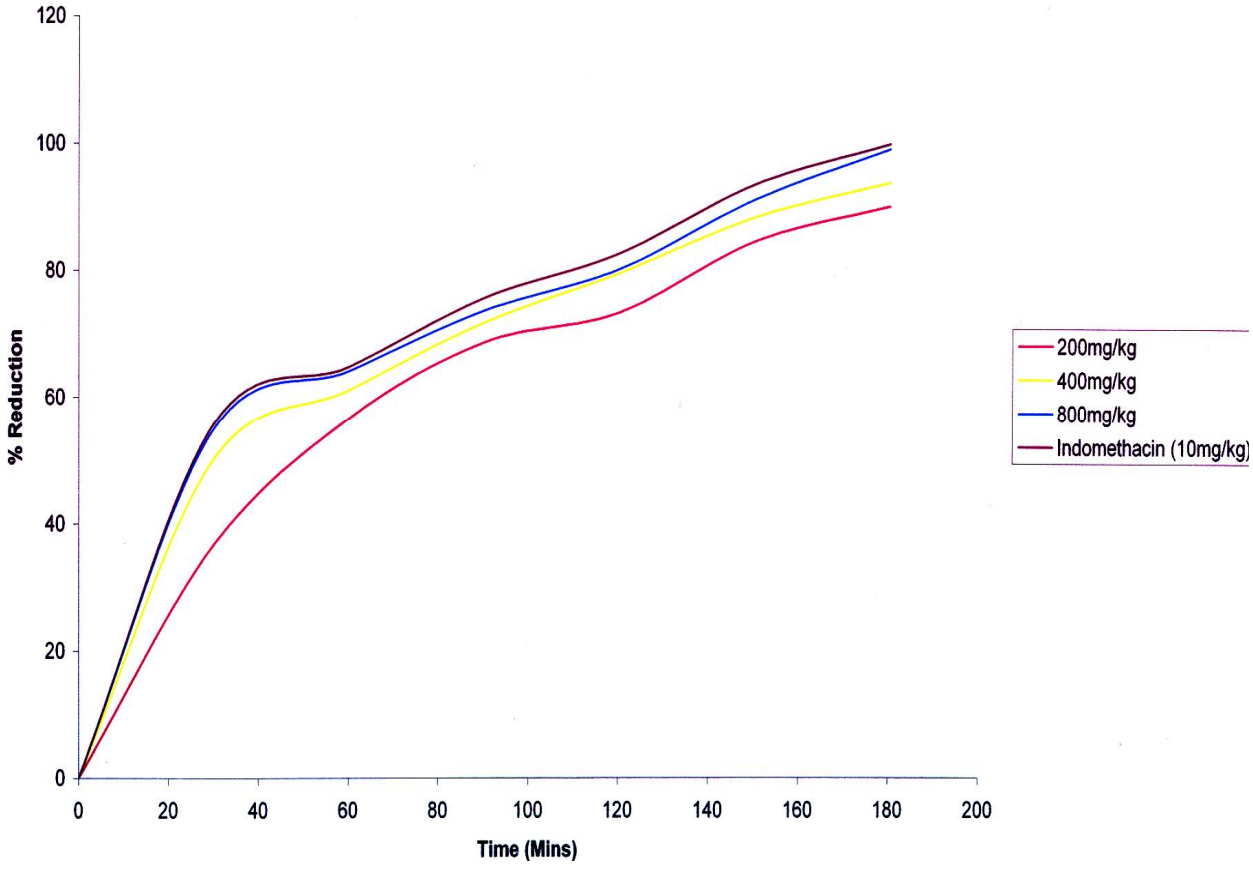


Fig. 4.11 **Effects of the ethanolic leaf extract of *Psidium guajava* on fresh egg albumen – induced paw oedema in rats**

Table 4.11 Effect of Ethanolic Leaf Extract of *D. glomerata* on Fresh egg Albumen - Induced Paw Oedema in Rats (n = 8; Mean ± SEM)

		Time in minutes and paw Oedema (in mm)					
Treatment	**Dose (p.o.)**	30 Min.	60 Min.	90 Min.	120 Min.	150 Min.	180 Min.
Distilled water (control)	5ml/kg	9.86±0.25 (mm)	11.08±0.47 (mm)	13.03±0.24 (mm)	13.80±0.16 (mm)	14.39±0.15 (mm)	1.478±014 (mm)
DGE	200mg/kg	6.46±030 (34.5%)	5.06±0.16* (54.3%)	4.20±0.19** (67.8%)	3.40±0.27** (75.4%)	2.68±0.08** (81.4%)	1.52±0.13*** (89.8%)
DGE	400mg/kg	4.94±0.19* (49.9%)	4.58±0.14* (58.7%)	3.72±0.08** (71.5%)	3.17±0.13** (77.0%)	2.36±0.12*** (83.6%)	1.09±0.06*** (92.6%)
DGE	800mg/kg	4.80±0.18* (51.3%)	4.27±0.22* (61.5%)	3.50±0.14** (73.1%)	2.80±0.19** (79.8%)	1.82±0.17*** (87.4%)	0.74±0.18*** (95.0%)
Indomethacin	10mg/kg	4.35±0.10* (55.6%)	3.91±0.13* (64.7%)	3.19v0.14** (75.5%)	2.42±0.08** (82.5%)	0.96±0.05*** (93.3%)	0.05±0.02*** (99.7%)

n = Number of animals; SEM = standard error of the mean;

* = $P<0.05$ = Significant difference compared with control values;

** = $P<0.01$ – Very significant difference; and

*** = $P< 0001$ very highly significant difference. Each quoted value represents the mean (± SEM) of eight observations. Inhibition of the egg albumen-induced inflammation by the plants extract and the reference drug used, indomethacin, are indicated as (percentage (%)

DGE = *Dichrotachys **glomerata*** extract

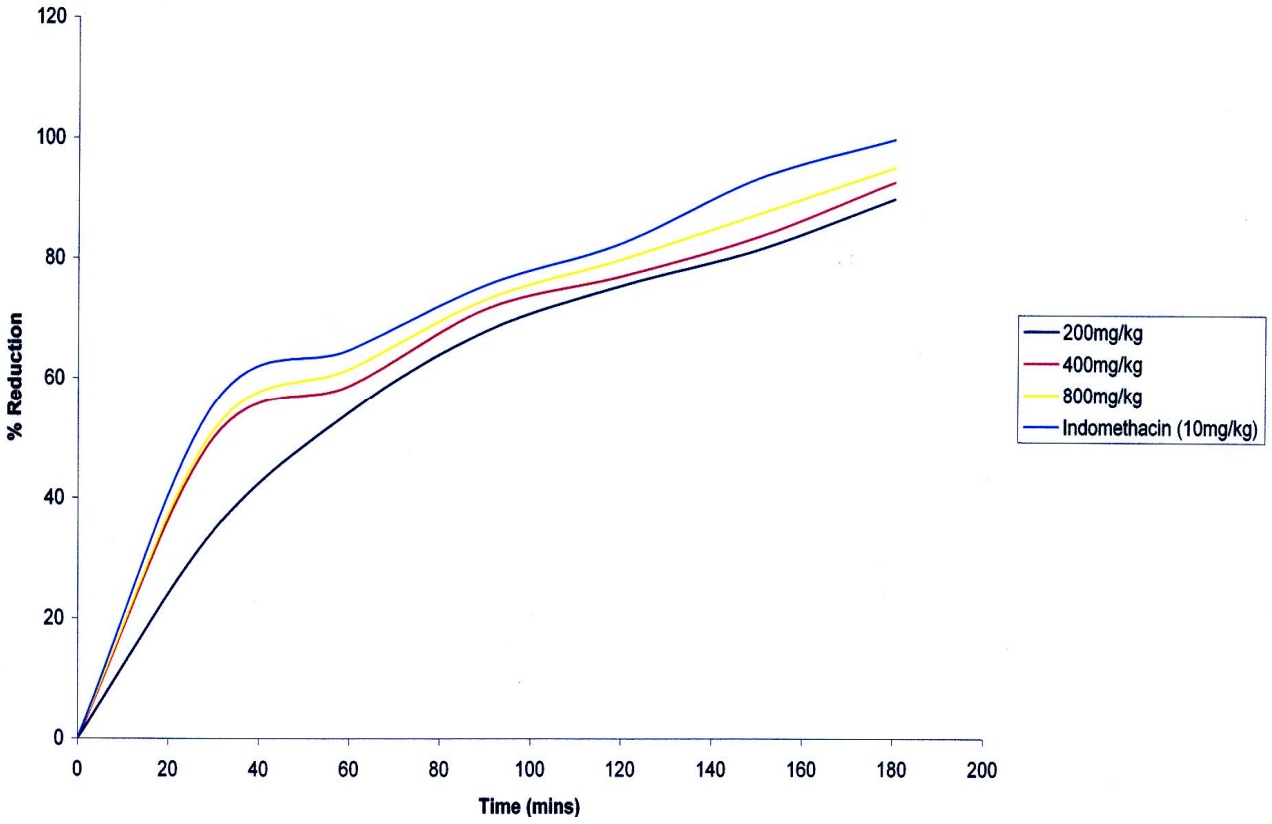


Fig. 4.12 **Effects of the ethanolic leaf extract of *Dichrostachys glomerata* on fresh egg albumen – induced paw oedema in rats**

4.1.11 Effect of the Extracts in Gastrointestinal Transit in Mice

The distance travelled by the charcoal meal (plug) in comparison to the entire length of the small intestine from the pyloric sphincter to the ileocaecum was used as index of gastrointestinal motility in mice. The ethanolic extracts of *P. guajava* and *D. glomerata* (100–400mg/kg) intraperitoneally (i.p.) significantly ($P<0.05$) reduced the gastrointestinal distance travelled by the charcoal plug in mice compared with normal saline (5ml/kg) as control (Tables 4.12 and 4.13). Both extracts (*P. guajava* and *D. glomerata*) produced a dose dependent decrease of gastrointestinal transit in mice.

4.1.12 Effects of the Extracts on Castor Oil – induced Diarrhoea in Rats

The ethanolic crude extracts of the leaves of *Psidium guajava* and *Dichrostachys glomerata* (100 – 400mg/kg *per os*) and loperamide 5mg/kg body weight, significantly ($P<0.05$) protected the rats against castor oil induced diarrhoea in a dose related manner when compared with the control (normal saline 5ml/kg) (Tables 4.14 and 4.15). The extracts and loperamide caused 60 – 100% protection of the animals against diarrhoea induced by castor oil. The maximal effect of the extracts was similar to loperamide.

Table 4.12 Effect of the Ethanolic Leaf extract of *D. glomerata* (DGE) on Mean[b] Gastrointestinal Transit in Mice

Drug	Dose (ip)	Distance Travelled by t Charcoal Plug
Normal saline	5ml/kg	66.36±6.50
DGE	100mg/kg	40.14±2.44*
DGE	200mg/kg	26.12±4.30*
DGE	400mg/kg	17.58±2.26*

b = mean ± standard error of the mean (SEM) based on 5 observations

* $P<0.05$ means significantly different compared with the control.

DGE = *Dichrostachys glomerata* Extract.

Table 4.13: Effect of the Ethanolic Leaf Extract of *P. guajava* (PGE) on Mean[b] Gastrointestinal transit in Mice

Drug	Dose (ip)	Distance Travelled by the Charcoal Plug
Normal saline	5ml/kg	71.52±5.80
PGE	100mg/kg	46.26±2.57*
PGE	200mg/kg	37.72±1.85*
PGE	400mg/kg	29.44±5.80*

b = mean ± standard error of the mean (SEM) based on 5 observations.

* $P<0.05$ means significantly different compared with the control.

PGE = *Psidium guajava* Extract.

Table 4.14: Effect of the Ethanolic Leaf Extract of *D. glomerata* (DGE) on Castor oil (0.5ml/Rat) – Induced Diarrhoea in Rats (n = 5; Mean ± SEM)

Treatment	Dose (p.o.)	Number of Mice with Diarrhoea	Protection (%)
Normal saline	5ml/kg	$^{5}/_{5}$	0%
DGE	100mg/kg	$^{1}/_{5}$	80%*
DGE	200mg/kg	$^{0}/_{5}$	100%*
DGE	400mg/kg	$^{0}/_{5}$	100%*
Loperamide	5mg/kg	$^{0}/_{5}$	100%*

* P. <0.05 All treatments reduced Castor oil- induced diarrhoea significantly.

DGE = *Dichrostachys glomerata* Extract

Table 4.15: Effect of the Ethanolic Leaf Extract of *P. guajava* (PGE) on Castor oil (0.5ml/rat) Induced Diarrhoea in rats (n = 5; mean ± SEM)

Treatment	Dose (p.o)	Number of Mice with Diarrhoea	Protection (%)
Normal saline	5ml/kg	$^{5}/_{5}$	0%
PGE	100mg/kg	$^{2}/_{5}$	60%*
PGE	200mg/kg	$^{1}/_{5}$	80%*
PGE	400mg/kg	$^{0}/_{5}$	100%*
Loperamide	5mg/kg	$^{0}/_{5}$	100%*

* $P<0.05$ = significant difference. All treatments are significantly different compared with the control (saline).

PGE = *Psidium guajava* Extract

4.1.13 Antibacterial efficacy of leaf extracts of *D. glomerata* and *P. guajava*

The Antibacterial screening of the ethanolic extracts of *D. glomerata* and *P. guajava* leaves, were carried out using pure cultures of *Staphylococcus aureus, Escherichia coli* and *Salmonella typhi*. The extracts (50, 100 and 200 mg/ml) showed significant ($P< 0.05$) inhibitory activity on the test organisms (Plates 8.0 – 10.0). Zone of inhibition of the bacterial growth ranged between 8.07 ± 1.0 - 16.07 ±0.09mm for *P. guajava* extract and 10.12 ± 0.05 – 20.0±0.09 mm for *D. glomerata* (Tables 4.16 and 4.17). The results of the inhibition of bacterial growth also showed that the extracts exhibited broad spectrum antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*, when compared with the distilled water (control). Their antibacterial effects were similar to those of ampicillin, a broad spectrum antibiotic, (Tables 4.16 and 4.17) used as the reference drug.

Table 4.16: Antibacterial Zone of Inhibition of Ethanolic Leaf Extract of *P. guajava* (N = 4; Mean ± SEM)

Concentration	Zone of Inhibition (mm)		
	Escherichia coli	***Staphylococcus aureus***	***Salmonella typhi***
200mg/ml	14.47±0.05*	17.50±0.08*	16.07±0.09*
100mg/ml	10.07±0.10*	14.05±0.06*	12.02±0.05*
50mg/ml	7.07±0.10*	10.06±0.05*	8.07±0.10*
Ampicillin 20mg/ml	12.07±0.10*	15.00±0.08*	15.0±0.10*
Distilled water	0.00±0.00	0.00±0.00	0.00±0.00

N = Number of observation SEM = Standard error of mean;

* P<0.05 compared with distilled water. All treatments were significantly different from distilled water. Each quoted value represents the mean (± SEM) of four observation of inhibition zones of the plants extract and the reference drug used in (mm).

Table 4.17: Antibacterial Zone of Inhibition of Ethanolic Extract of *D. glomerata* (n = 4; Mean ± SEM)

Concentration	Zone of Inhibition (mm)		
	Escherichia coli	***Staphylococcus aureus***	***Salmonella typhi***
200mg/ml	14.60±0.08*	16.50±0.10*	20.07±0.09*
100mg/ml	12.00±0.12*	14.00±0.08*	15.07±0.01*
50mg/ml	8.07±0.10*	10.10±0.08*	10.12±0.05*
Ampicillin 20mg/ml	10.10±0.68*	15.00±0.12*	14.02±0.05*
Distilled water	0.00±0.00	0.00±0.00	0.00±0.00

n = Number of observations

SEM = Standard error of mean;

- P<0.05 compared with distilled water. All treatments were significantly different from distilled water. Each quoted value represents the mean (± SEM) of four observation of inhibition zones of the plants extract and the reference drug used in (mm).

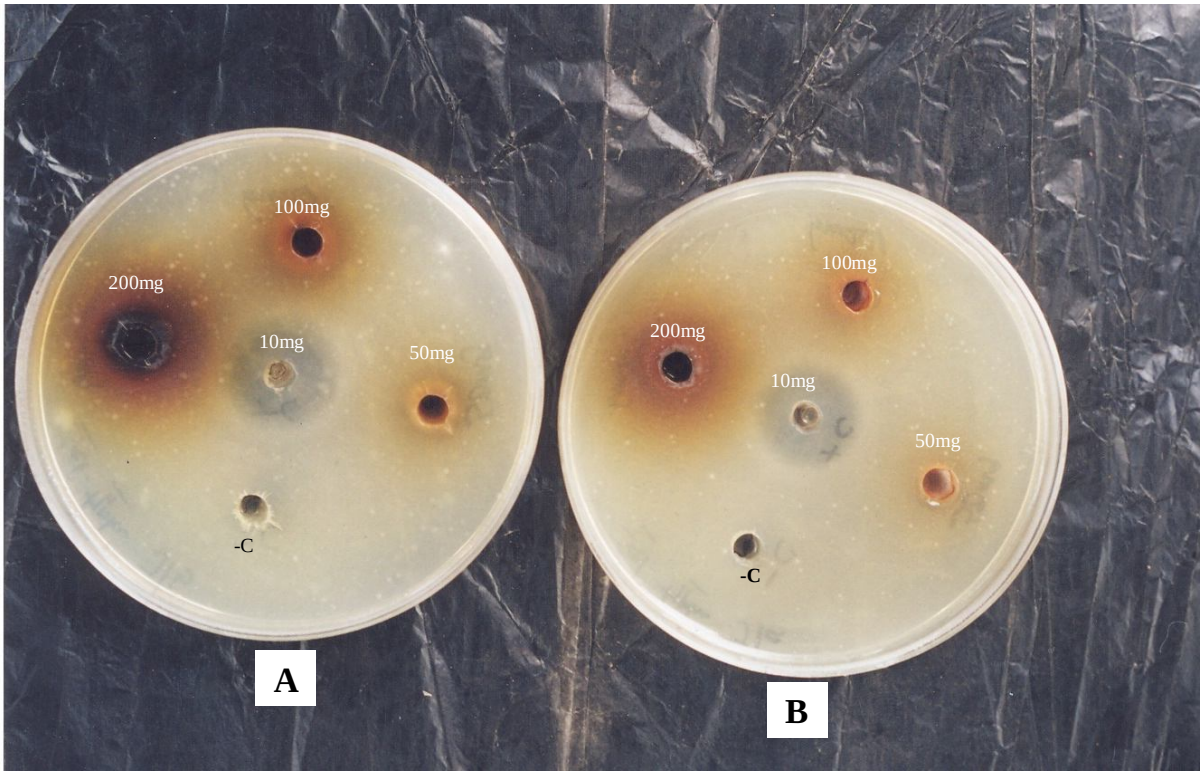


Plate 4.0: Inhibition of the growth of *Staphyylococcus aureus* by different concentrations of *D. glomerata* (A) and *P guajava* (B) ethanolic leaves extracts

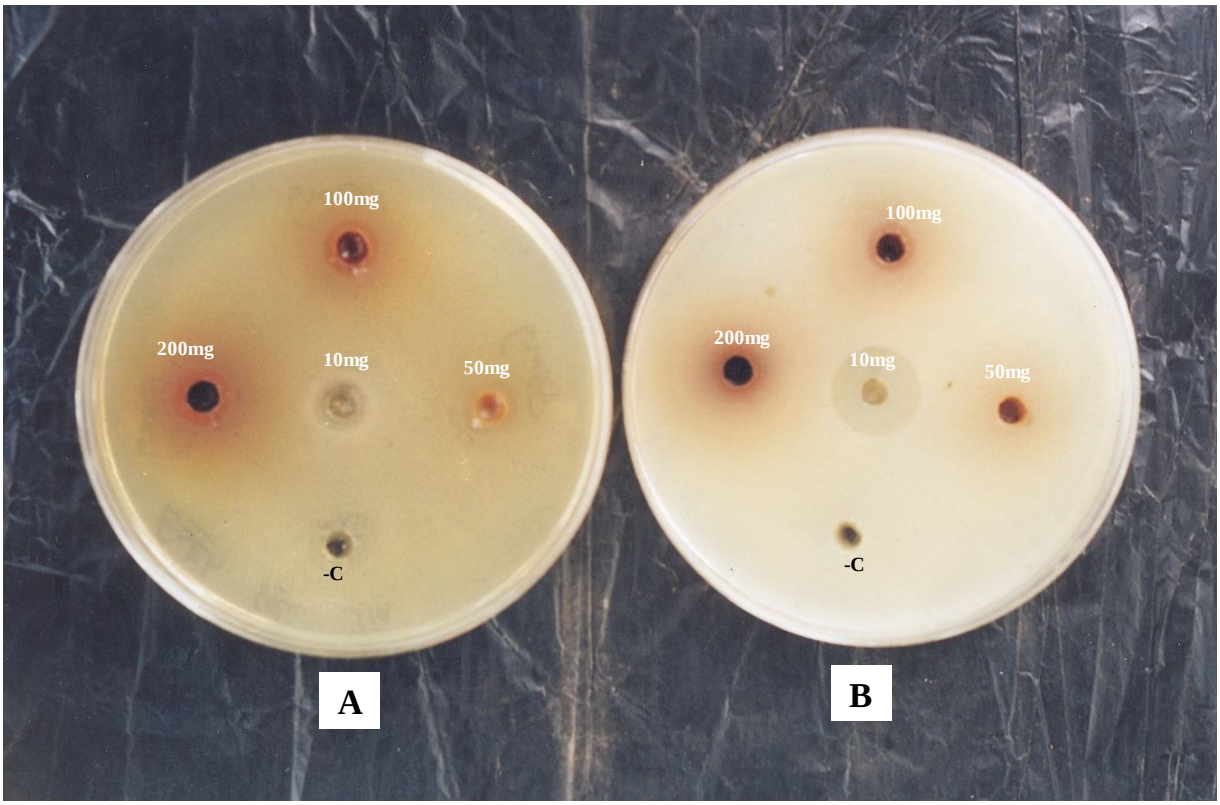


Plate 4.1: Inhibition of the growth of *Salmonella typhi* by Different concentrations of *D. glomerata* (A) and *P guajava* (B) ethanolic leaves extracts

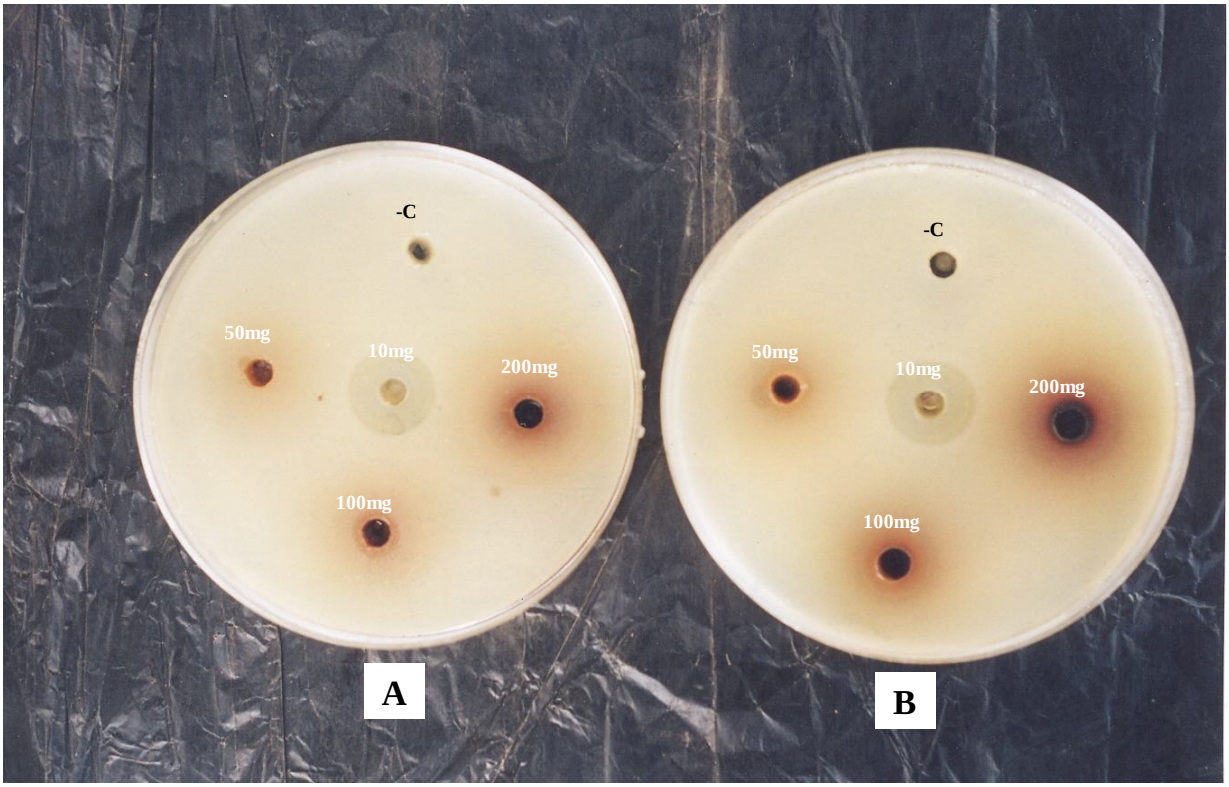


Plate 4.2: Inhibition of the growth of *Escherichia coli* by different concentrations of *D. glomerata* (A) and *P. guajava* (B) ethanolic leaves extracts

4.1.14 Anthelmintic effects of the extracts on faecal egg count

There were no signs of toxicity in all goats treated with the ethanolic extract of *P. guajava* and *D. glomerata*. The average rectal temperature of all the animals was within the normal range of 38 – 40^{o}C pre and post treatment. The crude extracts of *P. guajava* and *D. glomerata* (200 – 800mg/kg body weight) administered orally significantly (P<0.05) reduced the faecal egg count (FEC) per gram (EPG). The percentage reduction ranged from 38 – 91% in *P. guajava* and 33 – 81% in *D. glomerata* treated goats, respectively. The efficacy of the extracts against the helminthes increased in a dose dependent manner. The goats reduced their FEC from day 4 of extract administration and the reduction peaked on day 18 with 91% and 81% for *P. guajava* and *D. glomerata* leaf extracts, respectively. The reduction in faecal egg number was higher as extract dose increased. There was a progressive increase in faecal egg number in the untreated (control) groups. These results as shown by the faecal egg reduction produced by the extracts at 800mg/kg are comparable with that of albendazole, a conventional anthelmintic (Tables 4.18 and 4.19; Figs. 4.13 and 4.14).

Table 4.18: Faecal Egg Count Per Gram (EPG) and Percentage Reduction (%) of Faecal Egg Count for *P. guajava* leaf extract treated goats compared with untreated (control) group (Mean ± SEM; n = 5)

Day	No. of Animals	Group 1 Control (X103)	Percentage Reduction %	Group II 200mg/kg ($X10^3$)	Percentage Reduction (%)	Group III 400mg/kg ($X10^3$)	Percentage Reduction (%)	Group IV 800mg/kg ($X10^3$)	Percentage Reduction (%)	Group V Albendazole 25mg/kg ($X10^3$)	Percentage Reduction (%)
0	5	1.30±0.01	-	1.10±0.01	-	1.15±0.03	-	1.40±0.05	-	1.65±0.00	-
6	5	1.20±0.05	0.0	0.63±0.00*	38	0.45±0.02*	60	0.25±0.01*	82	0.15±0.04*	95
12	5	1.50±0.01	0.0	0.55±0.04*	53	0.35±0.01*	67	0.17±0.02*	89	0.08±0.02*	98
18	5	1.98±0.04	0.0	0.47±0.01*	57	0.32±0.04*	71	0.12±0.00*	91	0.00±0.00*	100
24	5	2.15±0.20	0.0	0.45±0.03*	59	0.31±0.04*	72	0.12±0.02*	91	-	-
30	5	2.50±0.00	0.0	0.55±0.02*	47	0.36±0.01*	68	0.14±0.00*	90	-	
36	5	2.6±0.10	0.0	0.60±0.01*	45	0.40±0.05*	64	0.15±0.00*	89	-	

n = Number of animals; SEM = Standard error of the mean

***** = P<0.05 = Significant difference between the treated and untreated (control) groups.

Table 4.19: Faecal Egg Count Per Gram (EPG) and Percentage Reduction (%) of Faecal Egg Count for *D. glomerata* leaf extract treated goats compared with untreated (control) group (Mean ± SEM; n = 5)

Day	No. of Animals	Group 1 Control ($X10^3$)	Percentage Reduction %	Group II 200mg/kg ($X10^3$)	Percentage Reduction (%)	Group III 400mg/kg ($X10^3$)	Percentage Reduction (%)	Group IV 800mg/kg ($X10^3$)	Percentage Reduction (%)	Group V Albendazole 25mg/kg ($X10^3$)	Percentage Reduction (%)
0	5	1.30±0.10	0.0	1.20±0.10	-	1.25±0.10	-	1.30±0.01	-	1.65±0.10	-
6	5	1.20±0.05	0.0	0.80±0.00*	33	0.60±0.03*	48	0.40±0.04*	69	0.15±0.04*	95
12	5	1.50±0.10	0.0	0.72±0.04*	40	0.50±0.01*	60	0.36±0.01*	72	0.8±0.00*	98
18	5	1.98±0.05	0.0	0.62±0.02*	46	0.45±0.01*	64	0.25±0.00*	81	0.00±0.00*	100
24	5	2.10±0.20	0.0	0.67±0.04*	44	0.42±0.02*	66	0.24±0.01*	81.5	0.00±0.00*	100
30	5	2.50±0.00	0.0	0.70±0.01*	42	0.47±0.01*	62	0.27±0.01*	79	-	-
36	5	2..6±0.10	0.0	0.72±0.04*	39	0.48±0.01*	62	0.30±0.10*	77	-	-

n = Number of animals; SEM = Standard error of the mean

***** **=** P<0.05 = Significant difference between the treated and untreated (control) groups.

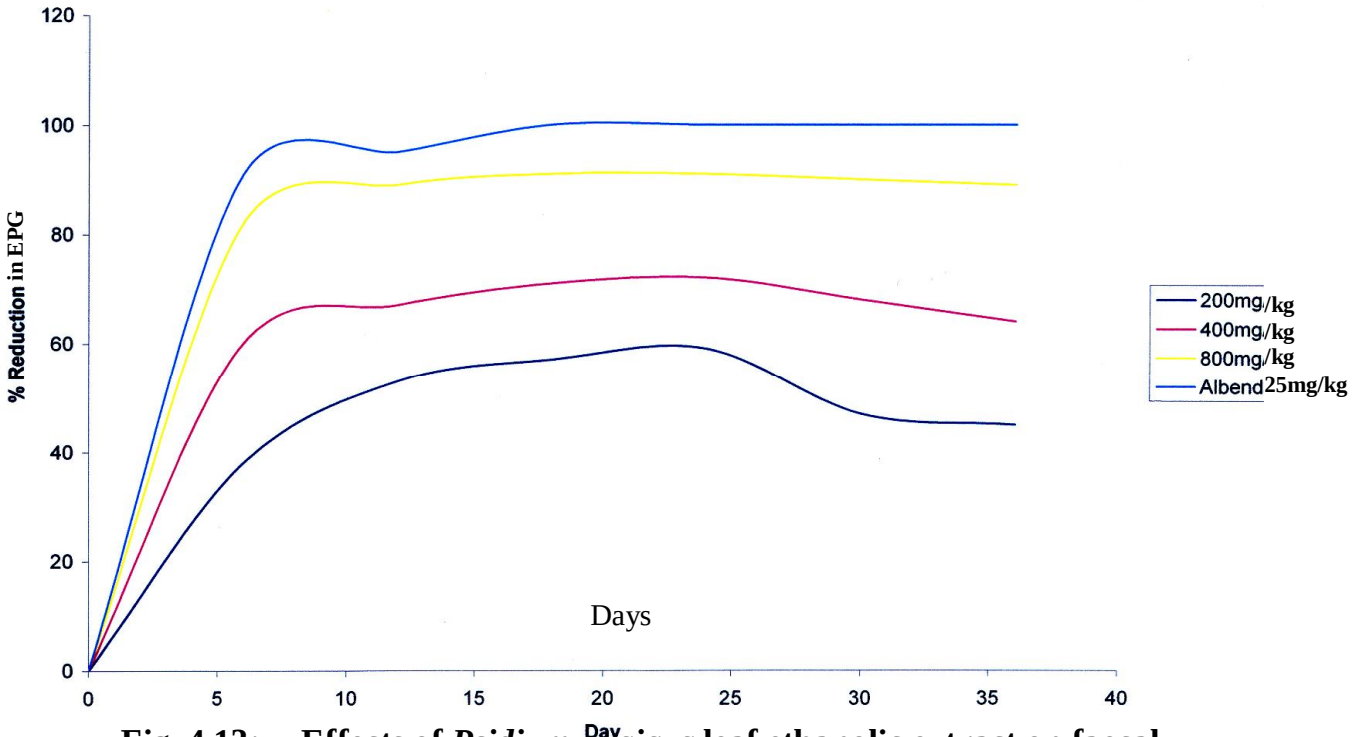


Fig. 4.13: Effects of *Psidium guajava* leaf ethanolic extract on faecal egg count per gram (EPG) compared with the control (albendazole)

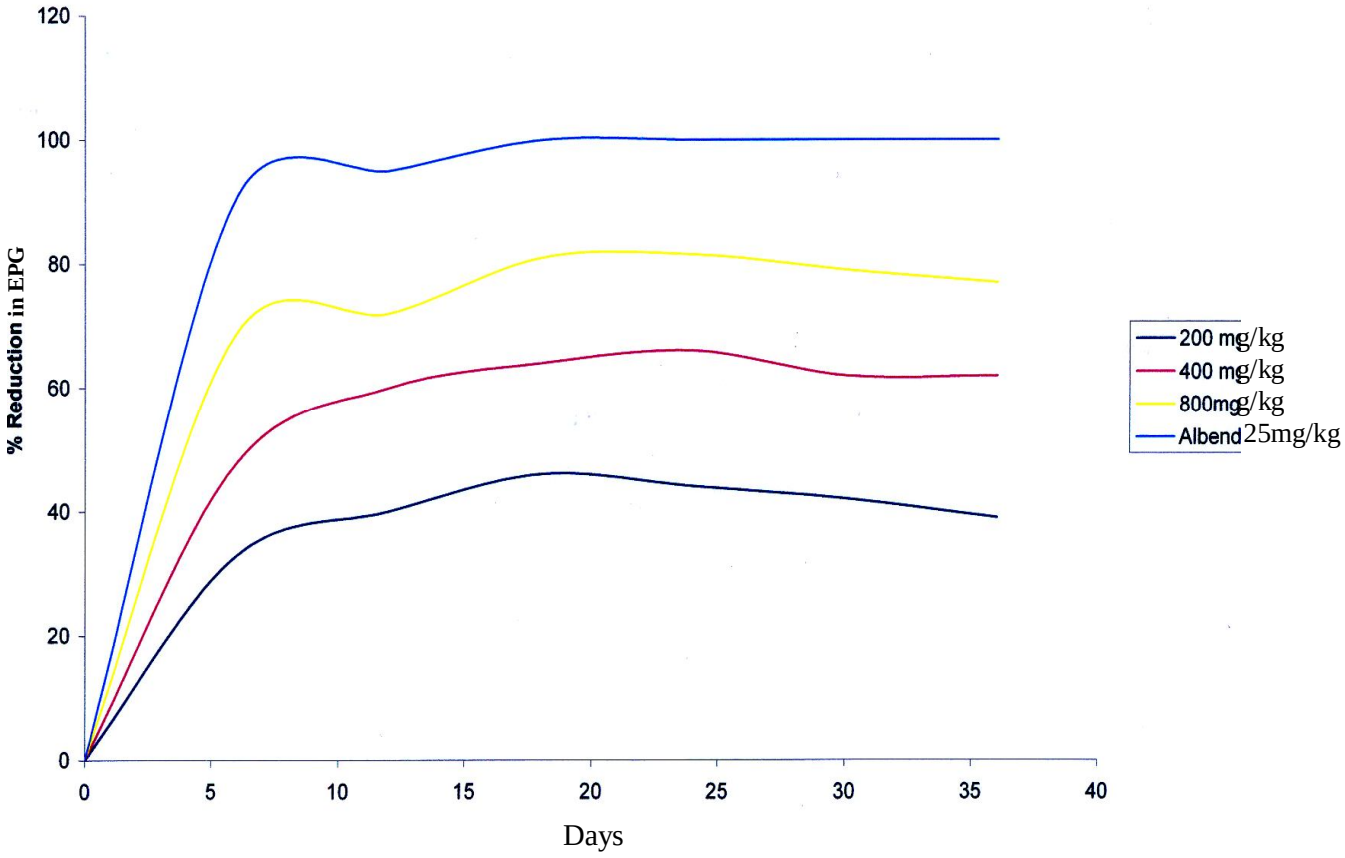


Fig. 4.14: Effects of *Dichrostachys glomerata* leaf ethanolic extract on faecal egg count per gram (EPG) compared with the control (albendazole)

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 DISCUSSION

Clinical signs of restlessness, inco-ordination (staggering), clonic convulsions (jerking), gasping (dyspnoea) and stupor (near unconsciousness), seen in the mice acutely poisoned with ethanolic leaf extractives of *Dichrostachys glomerata* and *Psidium guajava* were indicative of respiratory and nervous impairments. Poisoning with flavonoid rich plant extract is known to cause the above clinical signs in mammals as a result of respiratory impairment (Delwide, 1986; Marking, 1988).

The relatively high oral lethal dose, LD_{50}, of 1750±212mg/kg for *Psidium guajava* and 1,600±283mg/kg body weight for *Dichrostachys glomerata* ethanolic leaves extract, respectively, suggests that the plants extracts are relatively safe in mice.

Phytochemical analysis of the ethanolic leaves extracts of *P. guajava* and *D. glomerata* gave positive results for flavonoids, tannins, saponins, resins, carbohydrates and reducing sugars; glycosides and triterpenoids were also present in the extracts of *P. guajava* and *D. glomerata*, respectively. Flavonoids and tannins were highly positive in the two extracts. Flavonoids are a large group of naturally occurring compounds, found in fruits, vegetables, grains, bark, roots, stems, flowers, tea and wine. A variety of *in vitro* and *in vivo* experiments have shown that selected flavonoids possess anti-inflammatory activity (Menezes, 1998; Ogundipe *et al.,* 1998; Ahmadiani *et al.,* 2000; Chan *et al.,* 2001; Viana *et al.,* 2003).

Certain flavonoids possess potent inhibitory activity against a wide array of enzymes such as protein kinase C, Protein tyrosine kinase, phospholipase A_2 and others (Middleton and Kandaswani 2002; Viana *et al.,* 2003). Other flavonoids (Manthey, 2002, Viana *et al.,* 2003) potently inhibit prostaglandins, a group of powerful pro-

inflammatory signaling molecules. Studies have shown that this effect is due to flavonoid inhibition of key enzymes (i.e. lipooxygenase, phospholipase and cyclooxygenase) involved in prostaglandin biosynthesis. Inhibition of these enzymes provides the mechanism by which flavonoids inhibit inflammatory processes (Manthey *et al.,* 2002). Anti-inflammatory and antimicrobial effects have already been observed in tannins, saponins, and flavonoids (Ahmadiani *et al.,* 1998; Nuhu *et al.,* 2000). Okerulu and Chinwe (2001) reported the inhibitory effect on the growth of *Staphylococcus epidermidis, Streptococcus viridans, and Escherichia coli*.

Flavonoids, tannins and saponins were reported also to have inhibitory effect in the growth of *Bacillus subtilis, Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* (Ogundipe *et al.,* 1998).

Antispasmodic and antidiarrhoeal effects of an array of medicinal plants have been attributed to their rich flavonoid and tannin contents (Choudhury and Haruna, 1994; Haruna and Choudhury, 1997; Abdullahi *et al.*, 2001a, b and Oyewole, 2003).

The intestinal smooth muscle of rabbit jejunum have spontaneous rhythmic myogenic activities which are generated by pacemaker potentials for slow depolarization of the membrane markedly influenced by nerve activity (Koh *et al.,* 1998; Kuriyama *et al.,* 1998). It is the slow fluctuation (waves) that modulate the spike activity and spike frequency (Koh *et al.,* 1998). Slow waves are produced by specialized pacemaker cells located in the circular and longitudinal muscle layers of the intestine (Ward *et al.,* 2000; Taesotiku *et al*., 2003).

The results of the present studies demonstrated that the extracts of *P. guajava* and *D. glomerata* induced a graded relaxation of the smooth muscles of gastro intestinal tract (GIT), the severity of which depended on the concentration of the extracts. This was indicated by the progressive and significant decreases in both amplitude and frequency

of smooth muscle contraction. The results were consistent with the traditional use of these plants as natural anti-diarrhoeal agents, thus, confirming that the extracts are potent relaxants of GIT smooth muscle. Furthermore, the results demonstrated that the active principles contained in the plants' ethanolic extracts were capable of decreasing both the rate and force of contraction of smooth muscle as evidenced by the decrease in frequency and amplitude of contractions.

Besides, the extracts showed dose-dependent decrease in contractions similar to that obtained when the isolated tissue was treated with atropine alone. This again showed that the extracts exerted actions similar to atropine. Atropine is an established cholino-blocker (Satoh *et al.,* 2001). Some standard ant-diarrhoeal drugs are known to bring about their effects by decreasing GIT motility (Akah, 1996; Abdullahi *et al.,* 2001a, b). The results suggested that the extracts of *P. guajava* and *D. glomerata* have potentials of being good anti-diarrhoeal drugs and that the extracts induced relaxation may be mediated through complex mechanisms involving muscarinic cholinergic receptor sites.

From the myographic studies on the rabbit jejunum and guinea pig ileum, it was observed that the extracts antagonised ACh and histamine treated isolated smooth muscles which suggest that the relaxant action is on the GIT and supports anti-spasmodic actions mediated through muscarinic and histamine receptors. The extract significantly protected rats against diarrhoea evoked by castor oil in a dose-dependent manner. The maximal effects of the extracts of *P. guajava* and *D. glomerata* were similar to loperamide which is at present one of the most efficacious and widely employed anti-diarrhoeal drugs; loperamide effectively antagonised diarrhoea induced by castor oil (Niemegeers *et al.*, 1974; Abdullahi *et al.,* 2001a, b; Okpara *et al.,* 2006), prostaglandins (Karim and Adaikan, 1977) or cholera toxins (Farack *et al.*, 1981). The therapeutic effect of loperamide is believed to be due to its antimotility and antisecretory

properties (Couper, 1987; Abdullahi *et al.*, 2001a, b). From this present studies, it is likely that the extracts may mediate their effects through similar mechanisms.

The passage of a charcoal meal through the gastrointestinal tract in mice is used as parameter for intestinal motility and to study the laxative as well as the inhibition of intestinal motility (Abdullahi *et al.*, 2001a, b). The results on gastrointestinal transit revealed that the extractives of *P. guajava* and *D. glomerata* significantly reduced the gastrointestinal distance travelled by the charcoal meal.

Flavonoids, abundant in the extracts of *P. guajava* and *D. glomerata* used in this study have been demonstrated to inhibit contraction induced by spasmogens (Capasso *et al.*, 1988; Haruna and Choudhury, 1997; Abdullahi *et al.*, 2001a, b) and inhibit intestinal transit (Dicarlo *et al.*, 1993; Choudhury and Haruna 1994; Abdullahi *et al.*, 2001a, b). The ability of the extracts of *P. guajava* and *D. glomerata* to inhibit gastro-intestinal transit in mice suggests that the leaves of these plants possess antispasmodic agents. Properties such as these may underlie the observed antidiarrhoeal effect of ethanolic leaf extract of these plants (*P. guajava* and *D. glomerata*). The results provided some justification for the use of these plants as natural antidiarrhoeal agents and as remedy against colic.

The induction of diarrhoea with castor oil results from the action of ricinoleic acid formed by the hydrolysis of the oil (Watson and Gordun, 1962; Ammon *et al.*, 1974). Ricinoleic acid produces changes in the transport of water and electrolytes resulting in hypersecretory response. From the study, it is likely that the extracts of *P. guajava* and *D. glomerata* possessed antisecretory agent(s) and is likely, though not proved, to contribute to the anti-diarrhoeal potency of the extracts during infectious diarrhoea. Flavonoids abundant in the extracts of these plants (Table 4.6) have been demonstrated to inhibit intestinal secretion (Dicarlo *et al.*, 1993).

This work also highlighted the effects of *P. guajava* and *D. glomerata* extracts on acute inflammation in rats induced by fresh chicken egg albumen. Subplantar injection of fresh chicken albumen produced marked, sustained, time–related and progressive increase of hind paw oedema of the control, i.e. untreated rats. The results of the study indicated that ethanolic leaves extracts of *P. guajava* and *D. glomerata* significantly inhibited the fresh egg albumen–induced hind paw oedema in rats. This revealed that these extracts possessed anti-inflammatory effects on the mammalian experimental models used.

Indomethacin, a non-steroidal anti-inflammatory drug (NSAID) is commonly employed in the treatment and/or management of rheumatoid arthritis and osteoarthritis etc, (Viana *et al.,* 2003; Olaleye *et al.,* 2004) due to its anti-inflammatory and analgesic effects (Viana *et al.,* 1997). The drug reduces swelling and arthritic pain by inhibiting prostaglandin synthesis. Indomethacin has been reported to suppress inflammation induced by various phlogistic agents in experimental animal models (Viana *et al.,* 2003; Olaleye *et al.,* 2004). The results of this study tend to suggest that, like indomethacin, the extracts of *P. guajava* and *D. glomerata*, apparently, produce anti-inflammatory effects by inhibiting the synthesis and release of inflammatory mediators, including prostaglandin and histamine. Flavonoids and tannins, abundant in the *P. guajava* and *D. glomerata* extracts have been shown to inhibit lipooxygenase, phospholipase and cyclooxygenase – the key enzymes involved in prostaglandin biosynthesis (Manthey *et al.,* 2002; Viana *et al.,* 2003).

In this study, the results of the antibacterial screening show that the extractives of *P. guajava* and *D. glomerata* leaves possessed antibacterial activity. The antibacterial activities were quantitatively assessed by the presence or absence of inhibition zones. The zones were measured by the use of a transparent plastic rule. The maximal

inhibition zones for the bacterial strains (S*taphylococcus aureus, Escherichia coli* and *Salmonella typhi*) sensitive to the extracts were in the range of 13.0 – 20.0mm and 14.0 – 26.0mm for *P. guajava* and *D. glomerata,* respectively.

The ethanolic leaf extracts of *P. guajava* and *D. glomerata* were found to contain tannins, saponins and flavonoids in abundance (Table 4.6). These natural constituents were reported to have inhibitory effect on the growth of many pathogenic bacterial (Ogundipe *et al.,* 1998; Nuhu *et al.,* 2000; Ejimadu and Ogbeide, 2001; Okerulu and Chinwe 2001). It has been found that for *Enterobacterionaceae* to be regarded as sensitive to any antimicrobial agent, it must produce a zone of inhibition of greater or equal to 14.00mm (Barry and Thomsberry, 1985). The leaf extracts of *P. guajava* and *D. glomerata* are, therefore, effective anti-bacterial agents based on the Barry and Thomsberry theory. These collaborates the use of these plants as natural remedy for diarrhoea and other intestinal disorders. These results also showed that the extracts were dose-dependent in their action since less activity was observed at low concentrations. Ethanol was used for plant extraction in this study because previous studies reported that ethanol was a better solvent for more consistent extraction of antimicrobial substances from medicinal plants compared with other solvents such as water and hexane (Eno and Azah, 2004).

The results of the present anthelmintic studies show that there were no spectacular toxic signs in all the goats treated with the ethanolic extract of *P. guajava* and *D. glomerata*. The crude extracts of *P. guajava* and *D. glomerata* administered orally significantly reduced the faecal egg count (FEC) per gram (epg). The percentage reduction ranged from 38 – 91% in *P. guajava* and 33 – 81% in *D. glomerata* treated goats, respectively. The efficacy of the extracts against the helminths increased in a dose-dependent manner. The goats reduced their FEC from day 4 of extract administration and the reduction

peaked on day 18 with 91% and 81% for *P. guajava* and *D. glomerata* leaf extracts, respectively. The reduction in faecal egg number was higher as extract concentration increased. There was a progressive increase in faecal egg number from the untreated (control) groups. The observed anthelmintic activity of *P. guajava* and *D. glomerata* leaf extracts *in vivo* could be attributed to the presence of tannins in the plant extracts. Tannins are reported to have activity against plant parasitic nematodes (Taylor and Murant, 1966). The direct anthelmintic effect of condensed tannins from Quebracho® (an extract of *Schinopsis quebracho*) has been reported (Athanasiadon *et al.*, 2001). Enwerem *et al* (2001) also reported 50% deparasitisation of rats infected with *Nippostrongylus brasiliensis* after 100mg/kg tannins rich *Berlina gradiflora* extract was administered orally. These results are comparable with the effect of albendazole, a conventional anthelmintic. The extracts of *P. guajava* and *D. glomerata* could find application in anthelimintic therapy in veterinary practice. These findings lay credence to the use of these plants as natural remedy for diarrhoea-related diseases by traditional animal and traditional medicine practitioners in many communities of Plateau State, Nigeria. The results also support the use of these plants by local farmers in healthcare.

5.2 CONCLUSION

1. The oral median lethal dose (LD_{50}) of the crude ethanolic leaf extracts of *P. guajava* and *D. glomerata* in mice using distilled water as vehicle were 1750 mg/kg and 1,600 mg/kg, respectively. These doses could be regarded as relatively safe.

2. Flavonoids, tannins, saponins, resins, carbohydrates and reducing sugars were present in the extracts of these medicinal plants. The results suggest that vegetables are valuable not only for nutritional purpose but also as sources of medicine.

3. The extracts attenuated the spontaneous pendular contraction of rabbit jejunum and effectively blockaded the acetylcholine (8.0×10^{-2}µg/ml) – induced contraction of rabbit jejunum range (46.0 – 89%) and histamine (4.0×10^{-1}µg/ml) – induced contraction of guinea pig ileum (27.0 – 72.7%). These findings lend pharmacological support to the use of these plants as antispasmodic agents.

4. The extracts appear to have anti-diarrhoeal effects, since they inhibit the castor oil-induced diarrhoea and decreased the gastro-intestinal transit of charcoal.

5. The obtained results indicate that the ethanol extracts of *P. guajava* and *D. glomerata* leaves possess significant anti-inflammatory and analgesic properties following oral administration.

6. The extracts possess broad spectrum antibacterial properties that justify their use in folk medicine as anti-diarrhoeal agents

7. The extracts were also noted to possess some anthelmintic effects through the reduction of the faecal egg count in infected goats.

5.3 RECOMMENDATION

Further work is recommended to isolate and characterise the secondary metabolites responsible for the biological activities observed.

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APPENDICES

Appendix 1.1:

Faecal Egg Count per gram (epg) for *Dichrostachys glomerata* Leaf Extract Treated Goats (Day 0; Pre-treatment

Tag No Gp I	Gp II	Gp III	Gp IV	Gp V
DW 5ml/kg	200mg/kg	400mg/kg	800mg/kg	Albendazole 25mg/kg
A 1200	1200	1250	1550	1700
B 1550	1150	1300	1200	1650
C 1550	1250	1100	1500	1550
D 1050	1200	1350	1200	1700
E 1200	1200	1250	1050	1650
$\overline{X}$ 1300	1200	1250	1300	1650

DW = Distilled water

GP = Group

Appendix 1.2:

Faecal Egg Count per gram (epg) for *Dichrostachys glomerata* Leaf Extract Treated Goats (Day 6; Post-treatment

Tag No	Gp I	Gp II	Gp III	Gp IV	Gp V
A	1400	700	500	500	100
B	1800	650	650	300	200
C	1250	900	600	450	50
D	2000	850	700	350	250
E	1200	900	550	400	150
$\overline{X}$	1250	800	600	400	150

Appendix 1.3:

Faecal Egg Count per gram (epg) for *Dichrostachys glomerata* Leaf Extract Treated Goats (Day 12; Post-treatment

Tag No	Gp I	Gp II	Gp III	Gp IV	Gp V
A	1450	750	500	350	-ve
B	1300	800	450	400	100
C	1400	700	600	350	50
D	2100	600	400	400	150
E	1250	750	550	300	100
$\overline{X}$	1500	720	500	350	60

-ve = Negative for helminths eggs

Appendix 1.4:

Faecal Egg Count per gram (epg) for *Dichrostachys glomerata* Leaf Extract Treated Goats (Day 18; Post-treatment

Tag No	Gp I	Gp II	Gp III	Gp IV	Gp V
	(DW 5ml/kg)	(200mg/kg)	(400mg/kg)	(800mg/kg)	(Alb (25mg/kg)
A	1900	600	450	250	-ve
B	2100	650	400	300	-ve
C	2050	600	400	150	-ve
D	1950	600	500	250	-ve
E	1850	650	500	300	-ve
$\overline{X}$	1980	620	450	250	0.00

DW = Distilled water -ve = Negative for helminths eggs

Alb = Albendazole

Appendix 1.5:

Faecal Egg Count per gram (epg) for *Dichrostachys glomerata* Leaf Extract Treated Goats (Day 24; Post-treatment

Tag No	Gp I	Gp II	Gp III	Gp IV	Gp V
A	2200	650	400	200	-ve
B	2250	750	400	250	-ve
C	2100	600	450	300	-ve
D	2150	650	400	200	-ve
E	2050	700	450	250	-ve
$\overline{X}$	2100	670	420	250	0.00

-ve = Nagative for helminths eggs

Appendix 1.6:

Faecal Egg Count per gram (epg) for *Dichrostachys glomerata* Leaf Extract Treated Goats (Day 30; Post-treatment

Tag No	Gp I	Gp II	Gp III	Gp IV	Gp V
	(D.W 5ml/kg)	(200mg/kg)	(400mg/kg)	(800mg/kg)	Alb(25mg/kg)
A	2350	700	450	300	-ve
B	2600	750	500	250	-ve
C	2450	650	350	300	-ve
D	2500	650	600	250	-ve
E	2500	800	450	250	-ve
$\overline{X}$	2500	700	470	270	0.00

DW = Distilled water -ve = Negative for helminths eggs

Appendix 1.7:

Faecal Egg Count per gram (epg) for *Dichrostachys glomerata* Leaf Extract Treated Goats (Day 36; Post-treatment

Tag No	Gp I	Gp II	Gp III	Gp IV	Gp V
	(DW 5ml/kg)	(200mg/kg)	(400mg/kg)	(800mg/kg)	Alb (25mg/kg)
A	2600	800	500	300	-ve
B	2500	750	450	250	-ve
C	2700	600	450	300	-ve
D	2650	700	550	350	-ve
E	2500	750	450	300	-ve
$\overline{X}$	2600	720	480	300	0.00

DW = Distilled water -ve = Negative for helminths eggs

Appendix 2.1:

Faecal Egg Count per gram (epg) for *Psidium guajava* Leaf Extract Treated Goats (Day O; Pre-treatment)

Tag No	Gp I	Gp II	Gp III	Gp IV	Gp V
A	1200	1100	1050	1200	1650
B	1550	1000	2000	1300	1700
C	1500	1100	2050	1450	1550
D	1050	1200	1200	1500	1650
E	1200	1100	1200	1350	1700
$\overline{X}$	1300	1100	1150	1400	1650

Appendix 2.2:

Faecal Egg Count per gram (apg) for *Psidium guajava* Leaf Extract Treated Goats (Day 6; Post-treatment

Tag No	Gp I	Gp II	Gp III	Gp IV	Gp V
	DW 5ml/kg	200mg/kg	400mg/kg	800mg/kg	Alb (25mg/kg)
A	1400	650	400	250	100
B	1300	750	450	300	150
C	1250	550	700	150	100
D	2000	650	300	250	250
E	1250	650	400	300	150
$\overline{X}$	1250	650	450	250	150

Appendix 2.3:

Faecal Egg Count per gram (epg) for *Psidium guajava* Leaf Extract Treated Goats (Day 12; Post-treatment

Tag No	Gp I	Gp II	Gp III	Gp IV	Gp V
	DW 5ml/kg	200mg/kg	400mg/kg	800mg/kg	Alb (25mg/kg)
A	1450	550	350	150	50
B	1350	650	400	200	100
C	1400	500	300	200	100
D	2100	500	500	150	500
E	1300	550	200	150	50
$\bar{X}$	1500	550	350	170	50

Appendix 2.4:

Faecal Egg Count per gram (epg) for *Psidium guajava* Leaf Extract Treated Goats (Day 18; Post-treatment

Tag No	Gp I	Gp II	Gp III	Gp IV	Gp V
	DW 5ml/kg	200mg/kg	400mg/kg	800mg/kg	Alb (25mg/kg)
A	1900	450	300	100	-ve
B	2100	500	350	150	-ve
C	2050	350	250	100	-ve
D	1950	600	400	150	-ve
E	1850	450	300	200	-ve
$\overline{X}$	1950	470	320	120	0.00

-ve = Negtive for helminth eggs

Appendix 2.5:

Faecal Egg Count per gram (epg) for *Psidium guajava* Leaf Extract Treated Goats (Day 24; Post-treatment

Tag No	Gp I	Gp II	Gp III	Gp IV	Gp V
	DW 5ml/kg	200mg/kg	400mg/kg	800mg/kg	Alb (25mg/kg)
A	2200	450	250	150	-ve
B	2250	400	350	100	-ve
C	2050	500	300	100	-ve
D	2100	500	350	150	-ve
E	2150	400	300	100	-ve
$\bar{X}$	2150	450	310	120	0.00

-ve = Negative for helminths eggs

Appendix 2.6:

Faecal Egg Count per gram (epg) for *Psidium guajava* Leaf Extract Treated Goats (Day 30; Post-treatment

Tag No	Gp I	Gp II	Gp III	Gp IV	Gp V
	DW 5ml/kg	200mg/kg	400mg/kg	800mg/kg	Alb (25mg/kg)
A	2350	500	150	200	-ve
B	2600	700	550	100	-ve
C	2500	500	300	150	-ve
D	2450	500	450	150	-ve
E	2500	550	300	100	-ve
$\bar{X}$	2500	550	350	150	0.00

Appendix 2.7:

Faecal Egg Count per gram (epg) for *Psidium guajava* Leaf Extract Treated Goats (Day 36; Post-treatment

Tag No	Gp I	Gp II	Gp III	Gp IV	Gp V
	DW 5ml/kg	200mg/kg	400mg/kg	800mg/kg	Alb (25mg/kg)
A	2600	550	300	250	-ve
B	2500	600	500	100	-ve
C	2700	700	450	100	-ve
D	2650	650	350	150	-ve
E	2550	500	400	150	-ve
$\overline{X}$	2550	650	400	150	0.00

-ve = Negative for helminths eggs.

Appendix 3.1:

Effect of Distilled water (5ml/kg) in Fresh Egg Albumen Induced – Paw Oedema in Rats (n =8)

Treatment Gp		Time (in minutes and Paw Oedema in (mm) 30	60	90	120	150	180
1	Dose (5ml/kg p.o.)	9.6	10.6	13.1	13.6	14.3	14.7
2		9.8	10.5	13.2	14.0	14.5	14.8
3		10.0	11.5	12.6	13.8	14.2	14.6
4		9.8	11.4	13.4	13.9	14.2	15.0
5		10.3	11.6	12.8	14.0	14.5	14.8
6		9.6	11.5	13.0	14.1	14.5	14.9
7		9.7	10.6	13.1	14.0	14.3	14.8
8		10.1	11.0	13.0	13.8	14.6	14.6
$\overline{X}$		**9.86**	**11.08**	**13.03**	**13.80**	**14.39**	**14.78**

Appendix 3.2:

Effects of *Psidium guajava* Leaf Ethanolic Extract on Fresh Egg Albumen Induced Paw Oedema in Rats (n = 8)

Treatment	Dose (mg/kg p.o.)	Time (in minutes and Paw Oedema in (mm)					
Gp 2		**30**	**60**	**90**	**120**	**150**	**180**
1	200	6.5	5.0	4.2	3.8	2.5	1.6
2		6.0	5.1	4.0	3.6	2.5	1.4
3		6.2	5.2	3.8	3.2	2.6	1.5
4		6.2	5.0	4.0	3.8	2.5	1.5
5		6.3	5.4	4.2	3.6	2.7	1.4
6		6.5	5.0	3.8	3.2	2.5	1.5
7		6.0	5.0	4.0	3.1	2.6	1.6
8		6.6	5.4	4.1	3.6	2.7	1.4
$\overline{\mathbf{X}}$		**6.28**	**5.14**	**4.01**	**3.49**	**2.57**	**1.49**

Appendix 3.3

Effects of *Psidium guajava* Leaf Ethanolic Extract on Fresh Egg Albumen Induced Paw Oedema in Rats (n = 8)

Treatment Gp 3	Dose ((mg/kg P.O.)	Time (in minutes and Paw Oedema in (mm) 30	60	90	120	150	180
1	400	5.0	4.6	4.0	2.6	2.0	1.0
2		4.8	4.2	3.8	2.8	1.8	0.9
3		4.8	4.5	3.7	3.0	1.6	1.0
4		5.0	4.5	3.4	2.8	1.5	0.8
5		4.7	4.0	3.6	2.8	1.6	1.0
6		5.2	4.0	3.8	3.0	1.6	0.8
7.		4.9	4.5	3.5	3.0	1.8	1.2
8		5.0	4.2	3.7	2.8	1.5	0.8
$\overline{X}$		**4.90**	**4.31**	**3.70**	**2.85**	**1.70**	**0.94**

Appendix 3.4:

Effects of *Psidium guajava* Leaf Ethanolic Extract on Fresh Egg Albumen induced Paw Oedema in Rats (n=8)

Treatment Gp 4	Dose (mg/kg P.O.)	Time (in minutes and Paw Oedema in (mm) 30	60	90	120	150	180
1	800	4.4	4.0	3.5	3.0	1.4	0.2
2		4.5	4.0	3.6	2.8	1.2	0.2
3		4.4	4.2	3.5	2.4	1.3	0.2
4		4.5	4.0	3.4	2.8	1.2	0.1
5		4.6	4.0	3.4	2.7	1.1	0.1
6		4.4	3.7	3.5	2.8	1.2	0.2
7		4.5	4.0	3.4	2.5	1.4	0.1
8		4.4	4.0	3.5	2.8	1.4	0.2
$\bar{x}$		**4.46**	**3.99**	**3.48**	**2.72**	**1.28**	**0.16**

Appendix 3.5:

Effects of Indomethacin on Fresh Egg Albumen induced Paw Oedema in Rats (n = 8)

Treatment Gp 5	Dose ((mg/kg P.O.)	Time (in minutes and Paw Oedema in (mm) 30	60	90	120	150	180
1	10	4.2	4.0	3.0	2.4	1.0	0.10
2		4.2	3.8	3.2	2.4	0.9	0.00
3		4.4	4.0	3.1	2.5	1.0	0.01
4		4.3	4.1	3.3	2.4	1.0	0.00
5		4.4	3.9	3.2	2.4	1.2	0.11
6		4.3	3.8	3.0	2.5	0.9	0.00
7		4.5	4.0	3.4	2.3	0.9	0.10
8		4.2	3.7	3.3	2.5	1.0	0.00
$\overline{X}$		4.35	3.91	3.19	2.42	0.96	0.05

Appendix 3.6:

Effects of *Dichrostachys glomerata* Leaf Ethanolic Extract on Fresh Egg Albumen induced Paw Oedema in Rats (n = 8)

Treatment Gp 6	Dose (mg/kg P.O.)	Time (in minutes and Paw Oedemus in (mm) 30	60	90	120	150	180
1	200	7.0	5.0	4.2	3.6	2.6	1.5
2		6.5	5.2	4.6	3.8	2.6	1.4
3		6.2	5.0	4.3	3.5	2.7	1.4
4		6.3	4.8	4.0	3.6	2.7	1.6
5		6.2	5.0	4.0	3.4	2.8	1.5
6		6.8	5.2	4.2	3.2	2.6	1.6
7		6.5	5.0	4.1	3.0	2.7	1.5
8		6.2	5.3	4.2	3.1	2.8	1.6
$\overline{X}$		6.46	5.06	4.20	3.40	2.68	1.52

Appendix 3.7:

Effects of *Dichrostachys glomarata* Leaf Ethanolic Extract on Fresh Egg Albumen induced Paw Oedema in Rats (n = 8)

Treatment Gp 7	Dose (mg/kg P.O.)	Time (in minutes and Paw Oedema in (mm) 30	60	90	120	150	180
1	400	4.8	4.6	3.8	3.2	2.4	1.0
2		5.0	4.6	3.8	3.2	2.5	1.2
3		4.7	4.4	3.6	3.0	2.4	1.0
4		5.2	4.7	3.6	3.2	2.5	1.0
5		4.8	4.5	3.8	3.2	2.3	0.8
6		5.0	4.8	3.6	3.0	2.2	1.0
7		4.8	4.4	3.7	3.2	2.4	1.2
8		5.2	4.6	3.8	3.4	2.2	1.5
$\overline{X}$		4.94	4.58	3.72	3.17	2.36	1.09

Appendix 3.8:

Effects of *Dichrostachys glomerata* Leaf Ethanolic Extract on Fresh Egg Albumen induced Paw Oedema in Rats (n = 8)

Treatment Gp 8	Dose (mg/kg P.O.)	Time (in minutes and Paw Oedemus in (mm) 30	60	90	120	150	180
1	800	4.8	4.2	3.6	3.2	1.8	0.8
2		4.5	4.0	3.5	2.8	1.8	0.9
3		5.1	4.3	3.4	2.8	2.0	0.6
4		4.8	4.5	3.4	2.8	1.6	0.6
5		4.7	4.2	3.8	2.6	2.0	0.7
6		4.8	4.4	3.5	2.8	1.6	0.8
7		5.0	4.6	3.4	2.6	1.8	0.6
8		4.7	4.0	3.4	3.0	2.0	0.9
$\overline{X}$		**4.80**	**4.27**	**3.50**	**2.80**	**1.83**	**0.74**